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OBSERVATIONS ON *CHILOMASTIX INTESTINALIS* KUCZINSKI

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INTRODUCTION

Species of Chilomastix have been reported from a number of vertebrate hosts, viz., tadpole, fish, rat, goat, rabbit, guinea-pig and man. While species in this genus are based largely on characters such as size, cytostome, and differences in cysts, the species all possess morphological features which are so strikingly in common that they easily fall to the one genus. Among these common structural characteristics are the pear-shaped bodies of many of the trophozoites, the presence of a lipped cytostome with the cytostomal flagellum, the three anterior flagella, and the pyriform cyst.

It is worthy of note that the generic name Chilomastix has been adopted only recently to designate these species. This was largely brought about by a thorough revision by Chalmers and Pekkola (1917) of the work done within a period of a little over fifty years which resulted in reducing the number of tenable species from sixteen to seven, the other nine now standing only as synonyms of *Chilomastix mesnili* (= *Chilomastix davainei* Moquin-Tandon), the parasite of man. The name Chilomastix should stand as has been urged by Alexeieff (1912) in preference to Macrostoma as this name was originally used by Latreille (1825) for a mollusc, and also to Tetramitus, because of the absence of a trailing flagellum in Chilomastix.

The first record of species belonging to this genus is probably that of Davaine (1854) who reported from the motions of cholera patients during an epidemic in Paris, a parasitic flagellate under the name of *Cercomonas hominis* var. A. This species Moquin-Tandon named *C. davainei,* and Wenyon later described it as *Macrostoma mesnili.* Its correct name has been determined by Kofoid (1920) to be *Chilomastix davainei* (Moquin-Tandon). Alexeieff (1910) was the first to describe with considerable detail a species of this genus, calling it *Tetramitus caulleryi,* but in 1910 and 1912 he referred it to a new genus *Chilomastix.* This species is found as a lumen-dwelling parasite of the intestine of the tadpoles and axolotl.

Chilomastix intestinalis, the subject of this study, was found in the cecum of the guinea-pig and described very briefly by Kuczinski (1914) in his work of trichomonad flagellates. Being a parasite of an extensively used laboratory animal and related to the human parasite, it is thus not without general interest.

These investigations were carried out at the instance of Professor Charles A. Kofoid for whose help and criticism I am deeply grateful. I also wish to express my appreciation of the kind assistance rendered me in the laboratory by Dr. O. Swezy, and to both for access to their then unpublished drawings of *Chilomastix davainei* (see Kofoid and Swezy, 1920).

MATERIAL AND TECNIQUE

Chilomastix intestinalis was detected in 11% of the guinea-pigs examined during the course of the present work, excluding those animals which were artificially infected. The living forms were studied by placing a small amount of the intestinal contents mixed with a little normal saline solution on a slide, covering it with a coverslip and sealing it with vaseline. Care was observed not to dilute it with too much liquid so that too rapid a movement by the organisms could be avoided, and thereby the actual motions of the body could be watched with ease.

The Heidenhain iron-haematoxylin method of staining after fixation of the flagellates in Schaudinn's fluid was used in all cases for permanent mounts. The study of the cysts was facilitated by the employment of Kofoid's (1919) modified Donaldson's iodine-eosin stain. Chilomastix cysts readily became distinguishable in this mixture as pyriform bodies with the cytoplasm stained light pink and the cytostome standing out with clearness. With this stain also, a good definition of the flagella of the free forms is secured.

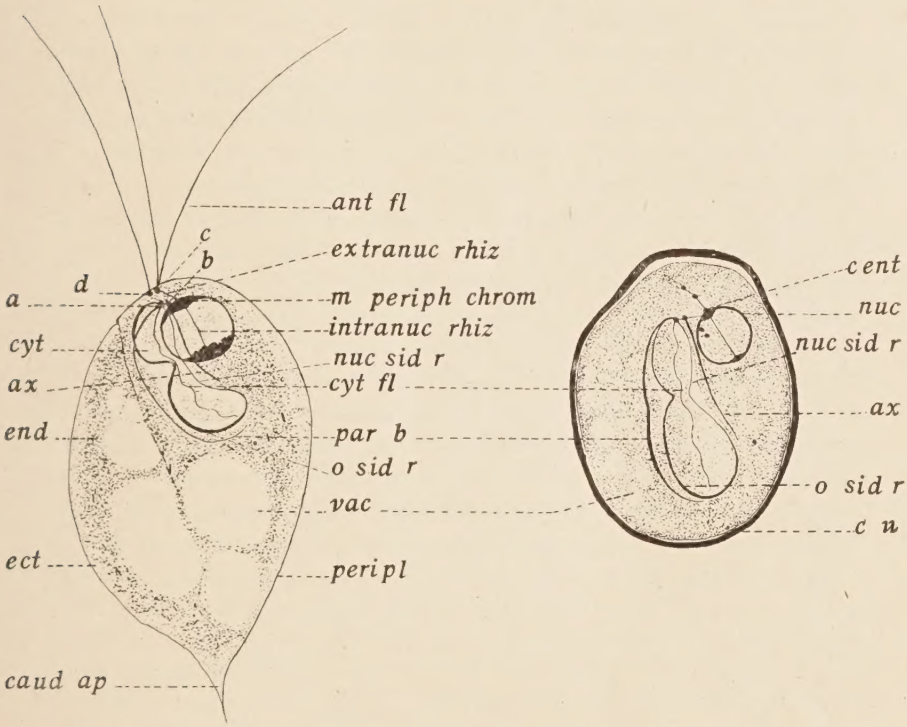
To determine the place of occurrence of the parasites the alimentary tract from the oesophageal opening of the stomach down to the rectum was taken out of the abdominal cavity and opened up with a pair of clean scissors at points about an inch apart, and material from each location was examined. This was done with two guinea-pigs which were found to be naturally harboring *C. intestinalis* and in two which were artifically infected.

From the results of the examinations it will be seen that, contrary to the findings of previous workers, this species is not exclusively a cecal parasite in the guinea-pig. In these hosts free and encysted stages of the flagellate were found beginning two inches above the ileo-cecal junction, though the infection is usually heaviest in the cecum. Extending from the cecum down to a distance of about 15 inches below it both stages of the parasite are encountered in fairly large numbers. It is only beyond this point where the fecal material begins to be less fluid

in consistency and harder fecal scybala are formed that cysts only are to be found. No evidence of tissue-invasion has been detected from the study of sections taken from various portions of the gut.

MORPHOLOGY OF THE FREE STAGES

The free form has been watched many times continuously for considerable time so as to determine the normal course of movement. It



A, ordinary trophozoite of *Chilomastix intestinalis;* B, cyst. × 3840. *a, b, c, d,* blepharoplasts; *ant. fl.,* anterior flagellum; *ax,* axostyle; *c w,* cyst wall; *cent,* centrosome; *caud ap,* caudal appendage; *cyt,* cytostome; *cyt fl,* cytostomal flagellum; *extranuc rhiz,* extranuclear rhizoplast; *ect,* ectoplasm; *end,* endoplasm; *intranuc rhiz,* intraneuclear rhizoplast; *m perph chrom,* massed peripheral chromatin; *nuc,* nucleus; *nuc sid r,* nuclear siderophilous rim of the cytostome; *o sid r,* outer siderophilous rim of the cytostome; *par b,* parabasal body; *peripl,* periplast; *vac,* vacuole.

has been seen that generally it travels not in a straight line but in a rather sinuous fashion with the anterior portion of the body undergoing a sort of spiral rotary motion. This is evidenced by the changing position of the cytostome which at one moment is on the left border of the organism, next on its right, to disappear as the ventral surface (i. e.

taking the surface where the cytostome is situated as ventral) is rotated dorsad. Sometimes this turning around of the anterior portion of the body with the posterior part following suite reveals a characteristic twist or spiral groove (Kofoid and Swezy, 1920) in some of these flagellates. This is apparent particularly in stained preparations in which there is seen in certain instances a spiral groove curving diagonally around the posterior part of the body (Fig. 5).

Metabolic movements of the body take place although not as marked as in trichomonad flagellates. A succession of diverse shapes may be seen in one individual such as the rounding out of a previously pointed posterior extremity (Figs. 1 and 2), or from a pear-shaped to a more rotund form. Irregular forms or even clubbed and elongated processes may thus be produced (Fig. 8). On the average the trophozoite is 12μ in length including the tail and 8μ in greatest diameter. Extremes in size have been encountered, as for instance, individuals measuring as long as 16.5μ and as short as 7.2μ (Fig. 1). Judging from the size of the latter it seems that it may have just emerged from the cyst. The nucleus is invisible in the fresh specimen, while the cytostome stands out with its prominent lips, with the posteriorly directed cytostomal flagellum undulating inside throughout its entire length.

Study of the stained specimens shows that there is rather definite ecto- and endoplasmic differentiation. The ectoplasm (*ect,* Fig. A) takes the stain lightly and is finely granular. The endoplasm (*end*) appears more heavily colored and its granulations coarser than those of the ectoplasm. The periplast (*peripl*), is the thin coating of the cell which is made up of differentiated ectoplasm and whose elasticity allows a certain degree of metabolic movements above described. Posteriorly it may be prolonged as a pointed caudal appendage which is free of the granular cytoplasmic material. The length of this process is variable, measuring on an average 4μ. Exceptionally long appendages (Fig. 4) may measure even 12μ. Tailless individuals with rounded posterior ends are encountered and they are not few (Figs. 2, 6). In the general run of cases the body takes on a variety of forms ranging from pear-shaped to those of oval or more rotund or of irregular contour. Vacuoles (*vac,* Figs. A, B) of various sizes are present and not infrequently they fill almost the entire endoplasmic area extending between the nucleus and that level of the body where it distinctly tapers down to form the caudal appendage. Bacterial masses form the common cell inclusions.

The nucleus (*nuc,* Fig. B) is situated close to the anterior margin of the body. While as a rule it is rounded in outline, oval forms are not rare. Overstained specimens show it to be a dense dark mass. How-

ever, if destaining is carried far enough, it becomes apparent that it consists of a heavy and firm nuclear wall of uniform thickness, and encrusted on the inner side, commonly at opposite poles, are the peripheral chromatin masses. Running between them a faintly staining line, the intranuclear rhizoplast (*intranuc rhiz*, Fig. A) can be seen on careful focusing. If only one peripheral chromatin block is present it is usually seen to lie on the anterior side of the nucleus (Fig. 3). Still another type of chromatin arrangement is that in which it is massed at the center of the nucleus as a karyosome (Fig. 10); or the chromatin may be broken up into small particles which lie scattered in the nucleus (Fig. 5). I was able to count as many as sixteen of these chromosome-like (?) bodies in one nucleus.

The cytostome and the structures immediately adjoining it have been in the past the subject of diverse interpretations, for the reason that not all of the component parts were identified so as to allow for the complete analysis of their relations in the organism. Heretofore, in the characterization of the different members of the genus Chilomastix, there have been described as invariably present a mouth opening or cytostome provided with rather prominent lips. Chalmers and Pekkola described in *Chilomastix mesnili* distinct siderophilous thickenings along the margins of the lips. Kuczinski's figures of *C. intestinalis* are very confused and vague, giving no clear analysis of the cytostomal region. Kofoid and Swezy (1920) working on the same species have fully analyzed this region and have found that in addition to those lines which are in the cytostomal rim, there are two other lines each running along the basal portion of the lips. They attribute to these two lines a parabasal and an axostylar (parastyle) significance respectively. In working over *Chilomastix intestinalis* from the guinea-pig I have found the same structures and they will now be described in this species.

The cytostome (*cyt*, Fig. A) is a lipped groove running from a level about the upper margin of the nucleus caudally and laterally to near the middle of the body. It measures on the average 5μ in length. Its breadth is quite variable since either approximation or drawing apart of the lips naturally modifies it. When the opening is fully patent it attains a width of 3μ. It is usually directed obliquely posteriorly with its opening wholly looking from one surface of the body. The shape is characteristic, the opening being composed of two wide spaces connected together by a narrow passage (Fig. 1). Such a figure is brought about by the curving middleward of the two sides of the lip with their convexities very closely approximated in some instances (Fig. 3), thus constricting the opening of the cytostome. The lower space is usually the rounder of the two. Of the two margins that on the side next to

the nucleus or the nuclear siderophilous rim (*nuc sid r*, Fig. B) generally appears less wavy and more lightly stained. They both originate from a common blepharoplast (*a*, Fig. A). From this blepharoplast there also arises the cytostomic flagellum (*cyt fl*, Figs. A, B) and a more or less heavily stained curved linear structure (*parab b*) which curves around the base of the outer lip. The latter organelle is the one considered by Kofoid as the homologue of the parabasal body as seen in other flagellates. At times it is quite elongated and follows the contour of the outer siderophilous rim (Fig. 2).

At the base of the lip proximal to the nucleus there runs a lightly staining line which has its origin from another blepharoplast (*b*, Fig. A). It curves around the base of this lip and seems to show in several instances a tendency to meet the parabasal body. This was called the parastyle and is supposed to correspond to the axostyle.

Besides the two blepharoplasts already referred to there are often encountered two others. One (*c*, Fig. A) gives a common origin to two anterior flagella, and another (*d*) gives rise to the third free flagellum. However, it happens not infrequently that the three anterior flagella take origin from a single blepharoplast (Fig. 2). In this instance there will therefore be only three blepharoplasts instead of the four which are of more common occurrence. Rarely there are three distinct blepharoplasts for the anterior flagella (Fig. 6). The blepharoplasts are connected with each other and the nucleus by means of the extranuclear rhizoplasts (*extranuc rhiz*, Fig. A).

ENCYSTED STAGES

Cysts of various dimensions have been encountered during the course of these examinations. In examining material from one host alone, multiple infection with more than one strain of the same parasite cannot be excluded. On the average the cyst measures 7.9μ along its long axis, and 7.2μ in its greatest transverse diameter. Very small cysts whose dimensions are about 5 by 3.8μ have been met with. On the other hand large ones measuring around 10.8 by 8.3μ also do occur.

The shape of the cyst is, as a rule, broadly pyriform, with one pole narrower than the other, with a slight, gradual constriction at about the junction of the anterior and posterior two-thirds. Spheroidal, oval and ellipsoidal form of cysts are not uncommon. There is a rather thick wall (*c w*, Fig. B) which is separated from the cyst contents by a space. This space is particularly evident at the anterior and narrower end of the cyst. In pyriform cysts the wall is uniformly thicker at the top than elsewhere. Within the cyst chromatoidal inclusions are frequently seen scattered throughout, sometimes as darkly staining ellipsoidal bodies (Fig. 7) and at other times as narrow chromatinic splinters (Fig. 11).

Diverse types of nuclear organization have been encountered in these cysts; some of them in all probability represent division phases.

There may be a nucleus with apparently no peripheral chromatin but with a distinct subcentral karyosome (Figs. 10, 12). This chromatin mass may appear divided (Fig. 14) into two; in which case it is brought about by the division of the centrosome, the daughter centrosomes traveling apart, drawing out a paradesmose between them. Nuclei with no central karyosome but with the peripheral chromatin encrusted at opposite poles are the ones commonly seen (*m periph chrom,* Fig. A; Fig. 11). The remarkable feature in most of these cysts, in addition to its shape, is the presence of the cytostomal or oral loop (*cyt,* Fig. A). The parabasal body (*Par b,* Figs. A, B) appears particularly more distinct in the encysted than in the free stages. The axostylar structure (*ax*) can be seen especially with well-oriented and with carefully differentiated cysts.

Evidence of mitosis, both in the trophozoite and in encysted stages has been searched for. None has been seen in the free forms while an unmistakable telophase was encountered in one cyst (Fig. 14). The chromatin material in the nucleus appears to be divided, with the centrosomes at corresponding poles outside the nucleus and connected with each other by a paradesmose. The mouth parts, the parabasal and axostylar structures are duplicated, a new set apparently arising anew from distinct blepharoplasts.

As in other flagellates, the well-regulated action of the different kinds of flagella may be attributed to the existence of an integrated, extranuclear organelle, the neuromotor system. Its component parts are the blepharoplasts which are connected to the nucleus by means of rhizoplasts, the parabasal and axostyle, the cytostomal and the free anterior flagella. It is remarkable that the parabasal and axostylar structures are both associated around the region of the cytostome, an actively movable part of the organism. They certainly in a measure contribute to the motor functioning of the oral aperture, interpreting the parabasal body as a kind of reservoir for motor activity of the neuromotor apparatus, and the axostyle as a modified intracytoplasmic flagellum.

Chilomastix intestinalis as a distinct species

Da Fonesca (1916), after Kuczinski (1914) first gave a brief account on this parasite, described it more fully as a separate species. Their published observations, however, seemed not to have convinced Chalmers and Pekkola (1917), and they regard it as similar to and the same species as the Chilomastix of man. In the light of the present work certain striking differences are noticed between the two forms, so

that one is forced to the conclusion that the Chilomastix of the guinea-pig should be considered as distinct species from that in man. Their differential characters may be tabulated as follows:

Criterion	*C. Intestinalis*	*C. davainei*
Size of the tropozoite...	12.2 by 8 μ (Da Fonseca) 13 to 16 μ by 7 to 9 μ	10 to 15 μ by 4 to 7 μ
Cystomal character	The external siderophilous rim has a deep, somewhat acutely pointed undulation mesiad. It takes the stain more heavily than the internal one	The external siderophilous has a wavy and gradual undulation. It is less heavily stained
Cyst	7.9 by 7.2 μ. Top of the cyst wider. Cyst less pyriform	7.5 by 6.5 μ. Top of the cyst more pointed. Cyst distinctly pyriform

SUMMARY

Observations were made on *Chilomastix intestinalis* Kuczinski from two naturally parasitized guinea-pigs and from two artificially infected ones.

The site of infection was determined to be in the lumen of the intestinal tract from about two inches above the cecum posteriorly to the rectum. The tropozoites were found to be cecal, partly intestinal, and partly colonic, lumen-dwelling parasites while the cysts occur at all levels but are more abundant posteriorly and in the fecal pellets. No evidence of tissue invasion could be found. A neuromotor system including the parabasal body and axostyle were described in *C. intestinalis,* similar to those described by Kofoid and Swezy (1920) for *C. davainei.*

No division phase has been encountered in the free forms while undoubted cases of apramitotic fission were seen in a cyst.

Chilomastix intestinalis of the guinea-pig is distinct from *C. davainei* of man. Its cysts have a broader, less constricted anterior region and are stouter and somewhat wider. The external limb of the cytostomal rim of *C. intestinalis* has a deeper, more acutely pointed, lateral constriction than the more gently undulating one of *C. davainei.*

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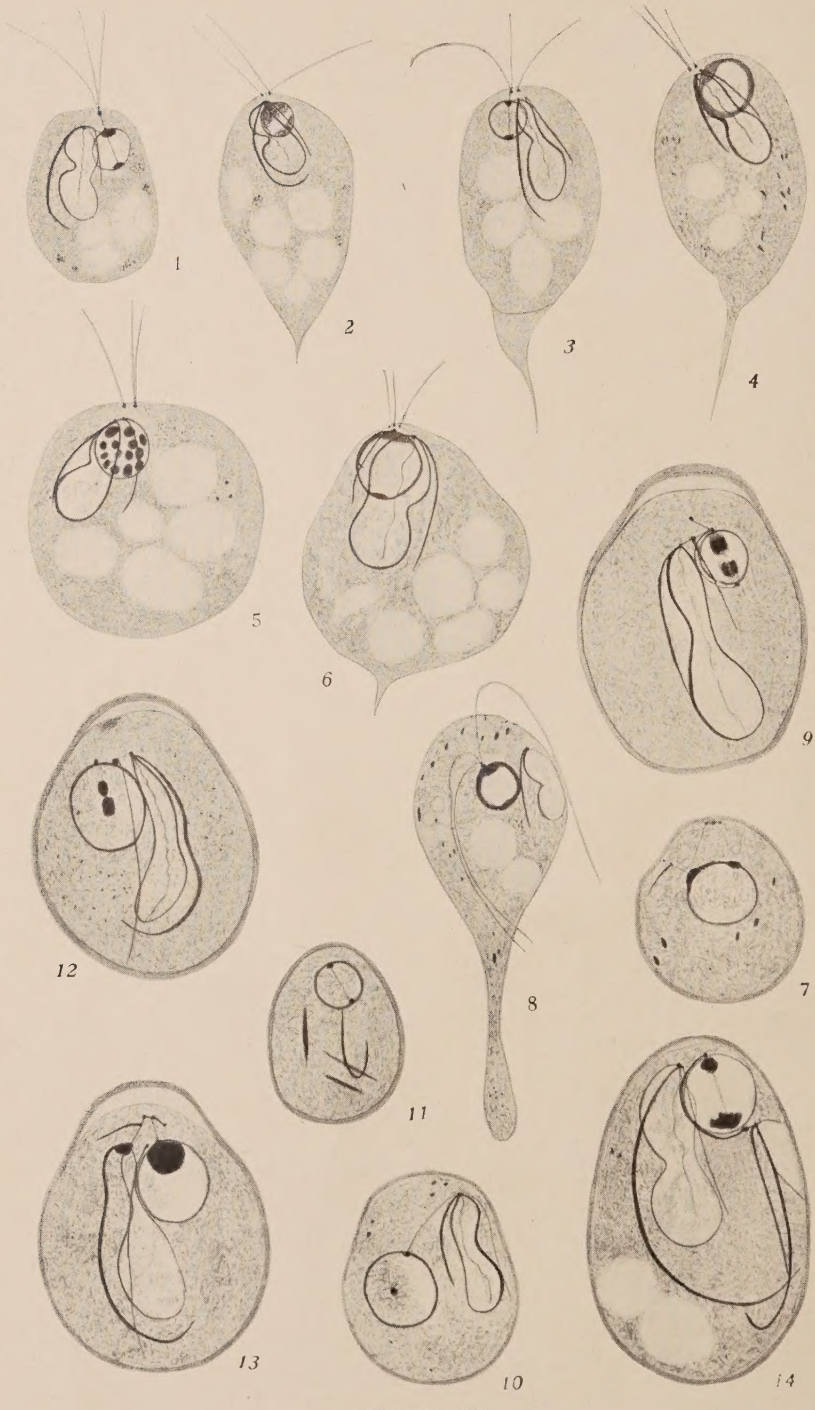

PLATE VI

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Explanation of Plate VI

Chilomastix intestinalis

All figures drawn with camera lucida from smeared preparations fixed in Schaudinn's fluid and stained in Heidenhain's iron-haematoxylin. × 4800, unless otherwise stated.

Fig. 1.—Small form, possibly just emerged from cyst. Trophozoite showing usual disposition of peripheral chromatin massed at opposite poles of nucleus. Note origin of anterior flagella from common blepharoplast. × 3200.

Fig. 2.—Trophozoite showing nucleus with great quantity of massed polar chromatin. × 3200.

Fig. 3.—Trophozoite showing spiral groove about posterior end; caudal area slightly above upper surface of rest of body. × 3200.

Fig. 4.—Free form with exceptionally long caudal appendage. Note peripheral distribution of chromatin on nuclear membrane. × 3200.

Fig. 5.—Rounded-up individual with two of its anterior flagella arising from a common blepharoplast; chromatin in form of small particles scattered throughout nucleus; endoplasm full of big vacuoles. × 3200.

Fig. 6.—Cytostome wide open, three anterior flagella take origin from three separate blepharoplasts. Possibly flattened in preparation. × 3200.

Fig. 7.—Small cyst of *C. intestinalis.* Massed peripheral chromatin in two blocks at the end of the nucleus, extranuclear rhizoplasts connecting nucleus with blepharoplasts; only trace of cytostome; chromatoidal inclusions present as oval, darkly staining bodies.

Fig. 8.—Form with clubbed caudal process produced by metabolic movement of body, or sloughing off of part of cytoplasm. × 3200.

Fig. 9.—Large cyst. Cytostomal margins stand out clearly; chromatin divided as in early anaphase; two centrosomes connected by paradesmose.

Fig. 10.—Encysted form with nucleus possessing central karysome.

Fig. 11.—Cyst with chromatoidal bodies in the form of splinters.

Fig. 12.—Parabasal body well developed. Chromatin appears to be undergoing division.

Fig. 13.—Cyst showing the connection of nucleus with blepharoplasts by rhizoplasts; single large mass of peripheral chromatin at one pole of nucleus.

Fig. 14.—A dividing encysted form. Chromatin mass is parted in anaphase; centrosomes at opposite poles, outside the nucleus, connected by extra-nuclear paradesmose. Both parabasals prominent; axostyle on right very distinct. Note elongated shape of cyst. Only part of other cytostome visible.

A COMMON INFUSION FLAGELLATE OCCURRING IN THE CAECAL CONTENTS OF THE CHICKEN

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During the past year a small flagellate has been regularly encountered in cultures from material containing eggs of *Heterakis papillosa,* a parasite of the common fowl, although this material was obtained from various sources. It has also been found in infusions contaminated with the feces of the rabbit. While this flagellate shows features somewhat characteristic of free-living organisms, its presence in the above cultures might be explained as well by contamination with fecal material as by the accidental introduction of a free-living species. In fact it has been found in large numbers in the caecal contents of young chickens which had been fed Heterakis material, so that it is evidently adapted to entozoic life. On the other hand it is found multiplying profusely in a variety of media outside the animal body. According to Alexeieff (1912), certain species of the genus Bodo are not only widely distributed as free-living organisms but may also occur as accidental parasites, retaining under such conditions the same characteristics as shown in their free state.

The flagellate under discussion is evidently very widely distributed. Similar organisms were found by Wight and Lucké (1920) in post-mortem cultures and smears from various organs of soldiers who died from influenza during the 1919 epidemic. The description and figures furnished by these authors do not coincide in every respect with our findings, possibly owing to differences in fixation and method of staining. On account of the variation indicated in the arrangement of the organelles and the body-outline, identification from their text is difficult but it appears probable that they dealt with the species here reported. In the present study also, great variation of form was noted in air-dried films, although the contour of the living organism is quite uniform. In attempting to identify this flagellate it has been found that descriptions of similar organisms are so incomplete and confusing that it seems best to describe the one in question somewhat in detail.

The organism was studied in fresh and stained preparations made from cultures at room temperature on "amoeba agar" (agar 14, sodium chloride 6, water 900), which proved to be the most satisfactory culture medium. It multiplied at 37.5 C on this medium, but did not persist

long in cultures kept at this temperature. It was also cultivated at room temperature in Locke-Ringer solution, but died in a few days unless vegetable or animal material was added to the solution, when it remained alive for a long time. No attempt was made to isolate it from the bacteria with which it grew. The structural details were studied in stained preparations, after killing the organism with osmic acid vapor and fixing it by the usual methods. The smears were stained by Giemsa's method and with Heidenhain's iron-hematoxylin, the latter giving the best results. A detailed description follows:

GENERAL MORPHOLOGY

The organism is colorless and transparent, more or less kidney-shaped and about twice as long as broad, with a variable length of from 5 to 12μ. In life the anterior flagellum is not readily visible; at first sight only the long, stout trailing flagellum is noticed. The former springs from the anterior extremity and the latter from a depression situated at the junction of the anterior and middle thirds of the body, dividing it into a smaller anterior and larger posterior portion. The surface from which the trailing flagellum emerges is called, for the sake of convenience, the ventral surface and is somewhat concave. The other, or dorsal surface, is sharply convex and regular. The anterior extremity is small and transparent, somewhat pointed and turned toward the ventral surface. This portion will be termed the "beak." It sometimes shows a clear space in the protoplasmic border between the points where the flagella emerge. The latter may be traced in their intraprotoplasmic course toward the dorsal surface, where they converge in the vicinity of a refractile body. The posterior portion of the organism is rounded and more voluminous than the anterior and it shows the nucleus situated near and slightly posterior to the ventral depression. The karyosome is sometimes readily distinguished as a large, refractile central mass. The posterior portion also contains numerous digestive vacuoles.

The ellipsoid or rounded nucleus is situated in the middle third of the organism, always nearer the ventral or concave surface and sometimes even slightly bulging from it. In well-differentiated preparations it is vesicular in type with a large, centrally-situated karyosome in the form of a very compact mass of structureless chromatin, and a thin limiting membrane with scanty peripheral chromatin. The space between the karyosome and the nuclear membrane is clear and shows no granules.

The cytoplasm in this species is a colorless, transparent and somewhat granular substance, containing numerous digestive vacuoles, which include food particles, mainly cocci and small bacilli. Some very refractile granules are seen in fresh preparations, situated near the

nucleus, in the space between it and the contractile vacuole. These granules take the chromatin stains, but are constant neither in position nor number.

The contractile vacuole is situated in the anterior third of the body, near the nucleus. It probably possesses an acid reaction as indicated by sensitized Neutral Red added to the culture. A very small, clear space was found in many of the living and fixed organisms, situated in the protoplasmic border of the "beak," just between the two points from which the flagella emerge (Fig. 6). Another vacuolar structure, possibly of the nature of a kinetonucleus, will be described later.

FLAGELLAR APPARATUS

The organism possesses two unequal flagella which arise separately from two indistinct granules which lie near a dorsally situated coarse mass of chromatin. Thence they cross the anterior portion of the organism to its ventral surface from which they emerge at some distance from one another. The more anterior flagellum, or tractellum, emerges from the anterior extremity; it is slender, short and never exceeds the length of the body. The trailing flagellum, or gubernaculum, is from two and a half to three times the length of the body and is considerably thicker than the anterior flagellum, especially at its base, where it is also quite rigid. It emerges from the ventral surface and is directed posteriorly.

At the origin of the flagella is a chromatin mass which lies against a clear, vacuolar space surrounded by a definite line, or ring, which takes the chromatin stain by both the Giemsa and Heidenhain methods; with the former it appears as a dull, pinkish mass, which often stains poorly, surrounded by a definite red ring; with the Heidenhain stain it shows either a lightly-stained substance, or merely a ring which is definitely darker than the contour of the ordinary vacuoles. This ring-like structure, which lies against the dorsal side (sometimes bulging from it as does the nucleus at the ventral side) may possibly represent the membrane of a kinetonucleus having a chromatin mass at its anterior pole. This interpretation must, however, be considered tentative for the present, as it is difficult to determine whether this ring is the demarcation line of the contractile vacuole, or a separate structure, for the organism suffers more or less change of form in stained preparations. Even with osmic acid fixation which gives good results in this particular case, the contractile vacuole varies from a median to a dorsal position. The peripheral position of the ring is suggestive of a contractile vacuole, as the latter (according to Minchin) is always an ectoplasmic organelle. Since the structure in question was never seen to contract, and because of its staining properties, its relation to the flagellar apparatus and its behavior during the process of division, there is much in favor of con-

sidering it as a true kinetonucleus. In the living flagellate, however, the contractile vacuole is always found in the anterior third, near the nucleus and very close to a bright granule from which the flagella seem to arise.

Hartmann and Chagas (1910) state that Bodo (Ehrenberg) has only one nucleus, in which the karyosome is connected by a rhizoplast with the basal granules from which the flagella arise and they consider the possession of a kinetonucleus and a special flagellar apparatus as characteristic of Prowazekia. On the other hand, Alexeieff (1912) states that the possession of a kinetonucleus is constant in Bodo.

MOVEMENTS

Under normal conditions when the organism is swimming free, it is impossible to follow it in its rapid motion through the microscopic field; but when the preparation begins to dry out, or when a viscid substance such as saliva is added to the culture, its movements can be followed and the flagella and their action readily studied.

The anterior flagellus or tractellum sweeps with broad undulations always toward the ventral side, moving the body by traction. The trailing flagellum or gubernaculum, which is rather stiff and thick at its base, extends posteriorly from the depression in the ventral surface in a slight curve, directed obliquely over its lateral surface from the antero-ventral to the postero-dorsal side (Figs. 1, 2). When the organism is at rest, attached by its trailing flagellum, the tractellum lies against the body, directed backward and parallel with the gubernaculum and at some distance from it. The latter, after leaving the immediate vicinity of the body, becomes gradually attenuated and exhibits broad undulations when the flagellate is in motion. At first glance the organism seems to progress in a spiral fashion, after the manner of the Rotatoria; but after careful examination it is seen that the rotation is incomplete and reversing in character. The combined action of both flagella gives to the flagellate a peculiar, semi-rotatory motion on its longitudinal axis combined with a lateral swaying about a point situated at the anterior extremity. Similar movements were described by Moroff (1904) in the case of *Bodo ovatus,* but the action of its anterior flagellum is quite different from that observed in our flagellate.

In some instances organisms were seen swimming slowly around a bacterial colony, with the dorsal surface toward the center of the circle described by their movements. Under these circumstances the anterior flagellum may be folded back against the surface of the body and is seen with difficulty. This peculiar motion seems to be due to the weak undulation of the tractellum together with a lack of control by the gubernaculum and is evidently abnormal, for organisms which showed this phenomenon died shortly afterward. When the flagellate is swim-

ming through the field its trailing flagellum occasionally becomes attached to the glass, to a bacterial colony or to some solid particle, thus anchoring it in position. It endeavors to free itself by means of violent springing or jerking movements and then lies quiescent; but the slightest disturbance causes renewed contractions, which are sometimes so rapid as to compare with vibrations. The organism jerks and pulls at the mass at short intervals until, at last, it may succeed in freeing its sticky appendage and continues on its way. In some cases it may swim slowly, carrying with it adhering material, but often fails to free its attached extremity, remains a prisoner, and dies. This is apparently due to the adhesive properties of the trailing flagellum under temporary, artificial conditions and cannot be considered as a special mode of life, as Kent (1882) has claimed for *Diplomastix* (*Bodo*) *saltans* (Ehrenberg).

INGESTION OF FOOD

No definite mouth or cytostome is found; food is taken in through the depression on the ventral surface of the organism, this process being aided by the movements of the tractellum. The process was studied in both fresh and fixed preparations from cultures into which carmin or Higgins' waterproof drawing ink had been introduced. It is rather remarkable that the ingested particles have a tendency to arrange themselves at first around the nucleus (Figs. 3, 4, 5), this localization being noted in many living and fixed organisms studied soon after they had been given carmin or ink. These particles later become scattered throughout the cytoplasm, being enclosed in vacuoles and later often accumulate in a mass near the posterior extremity. On one occasion, an organism expelled all the ingested ink and became clear, but the point at which it was ejected was not evident.

MULTIPLICATION

This species multiplies by binary division (Figs. 13-17). The earliest stages noted showed three flagella and the kinetonucleus in the process of division. Later stages of division were seen in organisms that showed four flagella arising from two kinetonuclei (red rings with Giemsa's stain) or from granules very close to them. The kinetonucleus must divide very rapidly, for we could find no intermediate stages. When this body has already divided, the true nucleus is still single or in process of division. After the division of the kinetonucleus the nucleus becomes elongated and shows at its center indefinite masses of chromatin (Fig. 14). Pale, diffusely stained masses next appear at its poles, the karyosomic chromatin becomes arranged at the center and, when the nucleus is stretched out, this mass of chromatin assumes the appearance of two cones with their apices in contact and having at their bases the two rather clear unstained masses (Fig 15).

At the completion of nuclear division, a faint line is seen uniting the two newly formed nuclei (Fig. 16). Dividing organisms seem larger and more or less rounded. The fission of the cytoplasm begins at the flagellar extremity and progresses toward the posterior end, splitting the organism longitudinally (Fig. 17).

Small, rounded forms without flagella occur in old cultures, which may be interpreted as resting-forms, or "cysts" (Fig. 18).

CLASSIFICATION

It is evident that this flagellate belongs to the sub-order Monozoa, family Bodonidae and, according to Alexeieff's classification to the genus Bodo, which is defined by him as follows:

"Body ovate, form variable for different species, constant for the same species. Two flagella, one anterior, one recurrent, generally larger, arise from a small granule situated in front of a voluminous chromophile body (kinetonucleus). Main nucleus with large karyosome, contractile vacuole single, toward the anterior extremity, near kinetonucleus. Nutrition by englobing solid parcles. They are free living, ubiquitous, sometimes presenting cases of accidental parasitism."

Hartmann and Chagas (1910) on the contrary consider the possesion of a kinetonucleus in organisms of this family, as a characteristic of the genus Prowazekia. They gave for the genus Bodo Ehrg. a flagellar apparatus consisting of two basal granules from which the flagella arise; these granules are connected with the karyosome by means of a rhizoplast.

These two conflicting statements have been a matter of lengthy discussion that will not be entered into this paper. It appears preferable to classify the organism in question in the genus *Bodo* Ehrenberg until the structure described tentatively as a kinetonucleus is demonstrated as such; and also until the absence of kinetonucleus is established for the genus Bodo and its presence accepted as diagnostic of the genus Prowazekia.

The nuclear character of kinetonuclei in general is questioned by Janecki and Kofoid who prefers the term parabasal for structures of this type. Considering the variations of such structures even within a given species, the question may be raised as to their importance as generic characters.

References to organisms similar to the one here described, are found in the literature and it may be well to consider them from a comparative point of view.

Pleuromonas jaculans Perty (1852). This organism is closely related to ours if it is not identical. The only difference found is with respect to the ingestion of food, which in Pleuromonas is, according to Perty, by means of a dorsal vacuole. In our flagellate food particles are taken in through the ventral depression at the base of the gubernaculum. Kent makes the following statement concerning this species: "It is further by no means improbable that the type *Pleuromonas*

jaculans, upon which the genus has been founded, is identical with *Heteronema* (*Bodo*) *saltans* of Ehrenberg, and which in addition to exhibing similar leaping movements, appears under insufficient magnification to possess a single flagellum only." Thus while Perty's original description gives to Pleuromonas one single flagellum it probably possesses two as claimed by Kent (1880) and also later on by Conn (1905).

Anisonema grande Ehrenberg sp. differs from the species here described only in possessing "an oral aperture adjacent to the origin of the posterior flagellum and communicating with a short tubular pharyngeal tract," Kent (1882).

Bodo ovatus Moroff (1904). This flagellate differs from ours in that the nucleus is centrally situated and the flagella are totally different as regards position and length.

Diplomastix saltans Ehrenberg sp. first described by Ehrenberg as *Bodo saltans,* differs in possession of a bilabiated or notched depression in its anterior extremity, which is considered as a cystostome. Kent's description gives its dimensions as 15.6 by 28 μ, which are about twice those given by Conn for *Pleuromonas jaculans.* Its movements, as described by him, agree in the main with those of the species discussed in this paper, but its "sendentary habits" are notably absent in the latter.

The flagellate under consideration agrees most closely with the description of *Pleuromonas jaculans* Perty, as corrected by Kent and by Conn. While it appears probable that this is the species with which we are dealing, we cannot positively identify it as such on account of disagreement with respect to the manner of ingesting food. It is possible that Perty may have been in error concerning this question, but this assumption is unwarranted without a survey of all available related flagellates. The descriptions at hand suggest that various generic and specific names have been applied to single species and many descriptions are not only incomplete but probably also incorrect in certain respects. The confusion is such that any further classification of the flagellate under discussion will at present not be attempted.

The occurrence of this species in the caeca of chickens that had been fed with material containing it in large numbers indicates a condition of accidental parasitism, for it appears to be widely distributed and is found multiplying profusely outside of the animal body in a variety of media and at low temperatures.

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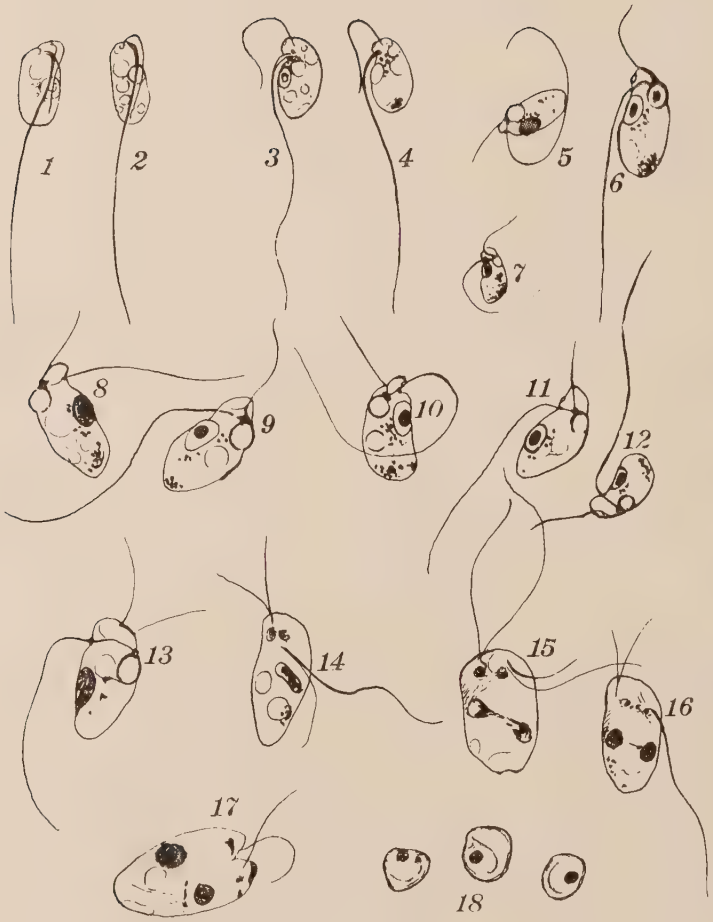

PLATE VII

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Explanation of Plate VII

Figs. 1, 2.—Fresh preparation. Organisms attached by gubernaculum.

Figs. 3, 4.—Fresh preparation, showing successive positions of flagella during locomotion. Ink granules at base of gubernaculum.

Fig. 5.—Distribution of granules around the nucleus (Heidenhain's iron hematoxylin stain).

Fig. 6.—Showing small vacuole at "beak" (Heidenhain's stain).

Fig. 7.—Very small organism, minimum size (Heidenhain's stain).

Figs. 8-12.—Showing relation of flagella to kinetonucleus (Heidenhain's stain).

Fig. 13.—Dividing form with three flagella (Heidenhain's stain).

Fig. 14.—Main nucleus begins to divide, when kinetonucleus is already divided in two red masses (Giemsa's stain).

Fig. 15.—Division of main nucleus, with formation of polar masses united by karyosomic chromatin (Heidenhain's hematoxylin).

Fig. 16.—Advanced stage of division of main nucleus (Heidenhain's stain).

Fig. 17.—Binucleated organism splitting into two (Heidenhain's stain).

Fig. 18.—Small aflagellar forms, suggesting resistant forms or cysts (Heidenhain's stain).

A NEW HUMAN TREMATODE FROM JAPAN*

William W. Cort and Sadamu Yokogawa

In the last ten years the Japanese have contributed very greatly to the knowledge of the trematodes of man. Some of this work has found its way into other languages in translations and abstracts. There are, however, many important investigations on the trematodes of man in Japan which are either entirely unknown outside of Japan or are known only in very brief and inadequate abstracts. We have been working for some time on a monograph on the trematodes of man. In connection with this work one of us (Yokogawa) has brought into English the recent Japanese investigations on this group. In this connection it was surprising to discover that a new human trematode of more than incidental occurrence, *Heterophyes nocens* Onji and Nishio, which had been described in 1915 (Onji, 1915, and Onji and Nishio, 1915) was entirely unknown outside of Japan.

The work of these authors on this species gains additional importance on account of the fact that they have not only described the adult but have solved part of its life history and shown the method of infection of the human host. Their work is the first light that we have on the life history or method of entrance into the final host of any member of the genus Heterophyes. The following account of *Heterophyes nocens* is taken from the Japanese papers cited above.

In 1910, Onji, a physician in a district of the Yamaguchi province (in the southwestern part of the main island of Japan) reported before the medical society of the province that he had found a peculiar type of trematode egg in a number of fecal examinations which he had made in his district. At this time he thought that the eggs might be those of the human liver fluke. Later he came to the conclusion that he had found a new human fluke and reported his findings in 1915, including an account of an encysted larval trematode in the muscles of a fresh water fish, *Mugil cephalus,* which he thought belonged to his new adult trematode, since this fish was frequently eaten raw by the people of the district. Later in the same year, in collaboration with Nishio, he described this trematode as a new species under the name *Heterophyes nocens* and gave an account of certain stages of its life history and of its method of entrance into man.

Heterophyes nocens Onji and Nishio is found in the middle part of the small intestine of man. It is known only from two villages, Onoda and Takachiho, in the Yamaguchi province of Japan. Its eggs were

* A contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

found in 31 out of 168 fecal examinations made from the inhabitants of these villages. In the intestine the flukes were found between the villi and sometimes attached to the mucous membrane near the bases of the villi.

The structure * of the adult of this species (Fig. 1) is similar to that of *Heterophyes heterophyes* (Looss) which has been known for many years as a parasite of man and other animals in Egypt. *H. nocens* is a very small trematode, having a length varying from 0.9 to 1.1 mm. and a width from 0.4 to 0.52 mm. The whole surface of the body is covered with very closely set scale-like spines. As in the other species of this genus, the genital pore is surrounded by a characteristic sucker, provided with a circle of chitinous rodlets. In *H. nocens* the number of these rodlets is about sixty. The following table shows the specific differences between *H. nocens* and the Egyptian species from man, *H. heterophyes*.

Characters	H. heterophyes	H. nocens
Size	1 to 1.7 mm. by 0.3 to 0.6 mm.	0.9 to 1.1 mm. by 0.4 to 0.52 mm.
Spines	With serrate posterior margins	Without serrate posterior margins
Oral sucker	0.09 mm.	0.08 mm.
Ventral sucker	0.23 mm.	0.20 to 0.22 mm.
Genital sucker	0.15 mm.	0.10 to 0.13 mm.
Rodlets on genital sucker	70 to 80	About 60
Intestinal ceca	Equal; end close to excretory bladder in extreme posterior of body	Unequal; one ending near anterior outer margin of posterior testis; and other close to excretory vesicle
Eggs	30 by 17 μ	28 by 15.5 μ

An examination of the table shows that these two species are distinct. *H. nocens* is smaller than *H. heterophyes*. The size of the suckers is less in the former and the genital sucker is smaller in proportion to the size of the ventral sucker. There are also differences in the number of rodlets on the genital sucker, in the character of the intestinal ceca, the eggs and the spines. Further a comparison of *H. nocens* with the analysis given by Ransom (1920) of the species of the genus Heterophyes shows that this form also differs from the species described in this genus from lower animals.

The Japanese authors have solved the question of the second intermediate host and the method of entrance into the final host of *H. nocens*. Onji and Nishio noted early in their study that the eating of raw fish, especially of the species *Mugil cephalus*, was a common

* A more detailed account of the structure and life history of this species will be included in the monograph mentioned above.

habit in the two villages where *H. nocens* were endemic. Examination of this fish showed the presence of a common encysted agamodistome which they took to be the larval stage of the species they were studying. This view was substantiated by feeding experiments on experimental animals. They were in these experiments able to follow the details of development from the encysted agamodistome to the adult.

The encysted stages (Fig. 3) were found in muscles and peritoneum of *Mugil cephalus*. The cysts are round to ellipsoidal in shape and have an average size of 0.163 by 0.136 mm. The wall of the cyst proper consists of two layers and there is a thin outer sac developed from the tissues of the host. The larvae (Fig. 4) freed from their cysts have an average size of 0.34 mm. by 0.15 mm. At this stage the ventral sucker and genital sucker are slightly back of the center of the body. The oral sucker has a diameter of 0.049 mm., the ventral sucker of 0.045 mm. and the genital sucker of 0.025 mm. It is interesting to note that at this stage the ventral sucker is distinctly smaller than the oral sucker. The pharynx has a size of 0.029 mm. by 0.026 mm. The excretory bladder is triangular in shape and filled with a mass of highly refractive concretions.

Experimental animals were infected with *H. nocens* by feeding them with fish containing these encysted larvae. The larvae grow rapidly in the final host, about doubling their size in three to four days. Eggs are present in the uterus five to six days after infection and they appear to be fully developed by seven or eight days. The reproductive organs were not distinguished by the Japanese authors in the encysted stage. They report, however, that after two or three days (Fig. 5) in the final host the testes, ovary, seminal vesicle and seminal receptacle can be distinctly distinguished and that the uterus appears as a very simply coiled tube. By three to four days the follicles of the vitellaria are clearly visible.

It seems very improbable that the two human representatives of the genus Heterophyes, *H. heterophyes* and *H. nocens,* have any injurious effects on man unless present in very large numbers. They are extremely small and apparently live more often free in the intestinal contents than attached to the wall. There is no evidence that they either break the intestinal mucosa, feed on blood or tissues, or produce a toxin. It must be borne in mind, however, that such organisms are truly parasitic and that under certain conditions of the host or when there is a very heavy infestation, they might produce injurious results. Also that a slight degree of injury to the host might be produced which would entirely escape notice.

These two species, however, have an importance to physicians working in Oriental countries since their eggs may be easily confused in

PLATE VIII

All figures concern *Heterophyes nocens*.

Fig. 1.—Adult. × 100. After Onji. Fig. 2.—Eggs. ×770. After Onji. Fig. 3.—Encysted agamodistome. × 100. After Onji and Nishio. Fig. 4.—Freed agamodistome. ×100. After Onji and Nishio. Fig. 5.—Immature stage two days after infection. × 200. After Onji and Nishio.

fecal examination with those of *Metagonimus yokogawai* Katsurada, an intestinal trematode of man in Japan, and the human liver flukes of the genus Clonorchis which have a wide distribution in the Far East. Both of these forms, especially the liver fluke, are definitely pathogenic and their accurate diagnosis is difficult except by the discovery of their eggs in the feces.

Since it is so easy to confuse the eggs of these small flukes, it is necessary whenever a small operculate egg is found in a fecal examination to study it very carefully with the high power of the microscope. With very careful examinations the eggs of *Heterophyes nocens, Metagonimus yokogawai* and Clonorchis can be accurately distinguished. The eggs of the first two species are the most difficult to separate since they have almost exactly the same size and shape. The egg of *Metagonimus yokogawai* is distincly yellowish in color and lighter than any of the other small fluke eggs. This light color is correlated with the fact that its shell is considerably thinner than that of *H. nocens* or *H. heterophyes*. Clonorchis eggs are narrowed toward the operculate end, and usually have distinct "shoulders" just back of the operculum while in the eggs of these other flukes the contour in optical section is almost regularly oval. Size differences are not reliable since the eggs are so nearly the same size and in each species there is a considerable range of variation.

Since the various species of the genus Heterophyes are not specific in their final hosts, and since as mentioned above their eggs are difficult to distinguish from other small fluke eggs, it seems probable that our present knowledge gives little real idea of their actual prevalence as human parasites. It is therefore important that medical men in regions where human flukes are found should be constantly on the lookout for these parasites. These considerations also suggest the importance of special training in medical zoology for physicians who are expecting to work in the Tropics or the Orient, where the diagnosis, treatment and control of diseases produced by animal parasites form a very important part of the routine practice.

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STUDIES ON MICROSPORIDIA PARASITIC IN MOSQUITOES

II. ON THE EFFECT OF THE PARASITES UPON THE HOST BODY *

R. KUDO

In a former paper (Kudo, 1921), I have described two new Microsporidia parasitic in mosquitoes collected at Urbana, Illinois. Due to the small number of infected insects found, I could not carry through the investigation.

During the summer of 1920, I studied several cases of microsporidian infections in mosquitoes in Pennsylvania and New York. Observation both in the field and in the laboratory brought to light imperfectly known points in the previous paper. In later papers I shall attempt to present the results of observations thus made. I am greatly indebted to Doctor H. G. Dyar of Washington, D. C., who knidly identified the species of mosquitoes dealt with herein.

OCCURRENCE OF MICROSPORIDIA IN MOSQUITOES

In the review of the literature dealing with the microsporidian parasites of mosquitoes of Europe, I (1921) failed to mention the cases recorded by Grassi and Ross.

Grassi (1901) speaks of two kinds of parasites in mosquitoes of the genus Anopheles. One of the forms was noticed in the general body cavity of the host. It was either free or attached to organs such as the intestine, salivary gland and dorsal vessel. In a probable young stage of the free form, it was a rounded multinucleate protoplasmic mass. On two occasions it contained oval bodies, each with a brilliant central corpusle and capable of escaping from the mass. But when the parasite is attached to the host organs which condition is more commonly found than the free state, it appears tubular or irregularly globular. It becomes surrounded by a membrane and the contents break up into a large number of spores each provided with a membrane. The presence of a polar capsule in the spore could not be determined. The second parasite invades especially eggs. In the protoplasmic mass one finds two, four and eight nuclei. A capsule is formed when eight sporozoites are produced in the mass. From this description and the figures given by Grassi, one cannot decide whether the said author saw Microsporidia or not. I have in vain tried to secure a statement and preparations from this investigator.

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* Contributions from the Zoological Laboratory of the University of Illinois, No. 190.

Ross (1906) states that he saw "spores of a protozoal parasite (in India in 1898), consisting of a collection of exactly eight spores closely packed within an oval envelope, which was probably the shell of the cell which contained the parent cytozoon. Each spore was oval, measuring 2 by 5μ, refractive, apparently hard, and containing a circular vacuole at one focus of the ellipse. Numbers of these little clusters of spores were found within the sheath of the ventral nerve trunk of the imago, especially in a small species of Stegomyia occurring near Ootacamund, and also, as far as I remember, in *C. fatigans* of Ootacamund itself. The mature spores appeared to escape in an unknown manner from the imago, and could be found in the water at the bottom of the vessel in which the insects were kept. There they seemed to grow much in size; and on several occasions I thought I observed amoebic embryos escaping from them. Since that time I have never seen these bodies again. They were not the same, I think, as the Myxosporidia with eight sporozites mentioned by Grassi, and also, apparently by Christophers and Stephens." Although no figures accompany the above quoted statement and the presence of a polar filament in the so-called spore is not spoken of, I am inclined to think that Ross probably saw a Microsporidian. It is of special interest that the nerve cord of an adult mosquito becomes infected by the parasites.

Quite recently three German authors reported briefly the occurrence of microsporidian parasites in the mosquitoes which they studied. Bresslau (1919) states that in a smear of the contents of body cavity of a larva of *Culex pipiens,* he found a microsporidian, *Nosema culicis,* the spores of which measure 4.5 to 5.5μ long by 1.8 to 2.4μ broad, and that he once saw another Microsporidian apparently closely related to the genus Thelohania in a larva of *Culiseta* (*Theobaldia*) *annulata.* Nöller (1920) figures stages in the sporogony of *Thelohania* sp., which were present in large numbers in the fat bodies of a larva of *Aëdes nemorosus* obtained in the vicinity of Hamburg in the spring of 1919. He did not find mature spores and expressed his view that it was probably identical with *Thelohania legeri* Hesse. The same author further states that *Nosema* sp. was found in the larvae of *Aëdes nomorosus* as well as *A. cantans* and that its somewhat broader shape suggested that it was not identical with *Nosema culicis.* Martini's valuable paper on mosquitoes (1920) does not contain any original observations except that *Nosema* sp. was found in *Aëdes* sp. in the vicinity of Hamburg, which is similar to the above quoted observation of Nöller.

The intensity of microsporidian infection in mosquitoes has not been given by European invstigators except Hesse (1904); it therefore would appear from the statement quoted in this and the former

reports that microsporidian parasites are either rare or very fragmentarily studied in European mosquitoes. In contrast with this, my observations during 1919 and 1920 show that the Microsporidian parasites are commonly found in mosquitoes in some parts of the United States.

The examination of mosquitoes of Pennsylvania and New York last summer revealed two facts among many other interesting matters: first, the microsporidian infection of mosquitoes takes a serious course, and second, Microsporidia occur rather widely among different species of mosquito on this continent.

The two Miscrosporidia, *Thelohania magna* and *T. illinoisensis*, which I described from Illinois, have been found to occur also in other localities. The former was found in *Culex territans* in Pennsylvania and the latter in *Anopheles qudrimaculatus* in New York. In the latter locality a new hitherto unrecorded Microsporidian was seen in larvae of *Culex apicalis*, to which the name *Thelohania opacita* nov. spec. is given.

GEOGRAPHICAL DISTRIBUTION OF MICROSPORIDIA IN MOSQUITOES

The following list includes all the microsporidian or Microsporidia-like parasites of mosquitoes of the world known up to the present:

Europe:		
	Thelohania legeri	Larvae of *Anopheles maculipennis;* France (Hesse, 1904).
	Thelohania sp.	Larva of *Culiseta annulatus;* Germany (Bresslau, 1919).
	Thelohania sp.	Larva of *Aëdes nemorosus;* Germany (Nöller, 1920).
	Nosema culicis	Larva of *Culex pipiens;* Germany (Bresslau, 1919).
	Nosema sp.	Larva of *Aëdes nemorosus* and *A. cantans;* Germany (Nöller, 1920).
	Nosema sp.	Larva of *Aëdes sp.;* Germany (Martini, 1920)
	Ambiguous forms:	
	Glugea (?) sp.	Larvae of *Culex* sp.; Germany (Pfeiffer, 1895).
	Nosema sp.	Host species not mentioned. Christophers and Stephens (1908).
	Glugea (?) sp.	Adult of *Anopheles* sp.; Italy (Grassi, 1900).
	Thelohania (?) sp.	Adults of *Anopheles* sp.; Italy (Grassi, 1900).
Asia:		
	Thelohania (?) sp.	Adults of *Culex fatigans* and *Stegomyia* sp.; India (Ross, 1906).
South America:		
	Glugea (?) *stegomyiae*	Larvae and adults of *Aëdes calopus;* Marchoux, Salimbeni and Simond (1903).
North America:		
	Thelohania magna	Larvae of *Culex pipiens* and *C. territans;* United States (Kudo, 1920, 1921).
	T. illinoisensis	Larvae of *Anopheles punctipennis* and *A. quadrimaculatus;* United States (Kudo, 1921).
	T. opacita	Larvae of *Culex apicalis;* United States (Kudo, 1921).

Of these writers, Hesse made an effort to study the effect of the parasite upon the host larvae, and suggested that the infected larva did not suffer from the infection.

The results of my own observations upon three North American Microsporidia are as follows:

Thelohania magna in *Culex territans*

A large number of *Culex territans* were found breeding in an old boat filled with rain water on Conewango Creek at Warren, Pennsylvania. Thirty-two larvae were collected on July 5, 1920. Microscopical examination showed that five of them were heavily infected by a species apparently identical with *Thelohania magna*. On July 10 more material was collected from the same place. Nine out of 193 living larvae and three out of 12 dead larvae were found to be heavily infected by the same Microsporidian. The infection was very much heavier than in the case of *Culex pipiens* and consequently the host animals showed a striking difference from the normal ones. In the case of *Culex pipiens,* I have stated (1921), that the larvae "did not show any marked difference in their activity" and that "some of the larvae appeared to be more opaque whitish, with more or less distended thorax, than the majority." The heavily infected larvae of *Culex territans* were uniformly much smaller in size than the normal ones. At first I thought that these smaller infected larvae hatched out from eggs which were laid later than those which gave rise to healthy ones. Yet I did not find normal larvae of smaller dimensions in the collected material. It seems probable that the decrease in size of the infected larvae is due to the presence of the Microsporidian. A similar condition is found in silk-worms, *Bombyx mori,* infected by *Nosema bombycis*. A single batch of eggs laid by a moth give rise to worms of variable size according to the degree of infection by the parasites. The difference in size between normal and infected worms is frequently so great that it is hard unless one traces the larval development to conceive that they hatched out from eggs laid by one and the same moth.

The opacity of the body of the infected larvae was striking. The heavy infection could easily be determined under a magnification of about 10 diameters by virtue of milky opaque and whitish color with a slightly yellowish tone. Although the infected larvae of *Culex pipiens* did not show any marked decrease in activity, several of the heavily infected larvae of *Culex territans* were far less active than the normal ones. When kept in an aquarium, they usually stayed on the bottom without any active movements. When they came up to the surface of the water, the head was almost always directed upwards and they stayed in this position at the surface until disturbed. When disturbed the normal ones swam down very swiftly, while the diseased ones

would start this motion very slowly. In a few seconds the normal larvae would come up again to the surface, while the heavily infected ones would not do so for some length of time.

On July 10 the debris on the bottom of the boat was collected and twenty-two dead larvae were found completely filled with the Microsporidian spores. Among various Protophyta and free living ciliates and flagellates, fragments of infected larval tissues and apparently normal ones together with scattered mature spores were recognized.

Rearing Experiment No. 1.—Thirty-six larvae from the collection made on July 5 were kept in a glass jar, the bottom of which was covered with garden soil. The dead larvae were subjected to daily microscopical examination for the schizonts and spores of the Microsporidian. On July 7 three larvae were found motionless on the bottom. One was entirely dead and showed no microsporidian parasites. The other two perfectly motionless when handled by a pipette, exhibited a very weak muscular contraction under the microscope. Both were heavily infected by young and mature sporoblasts and free spores in the adipose tissue and body cavity. The anal gills were filled with scattered spores which could be seen under a low magnification. On July 8 one larva was found dead. It was heavily infected and the body was completely filled with the spores, exhibiting the typical opacity. Later, two, nine, two, two and five larvae were found dead on July 9, 10, 11, 12 and 13, respectively; these did not show any degree of microsporidian infection. From the larvae seven pupae developed on July 8 to 10. In two of them it was found that one spore was attached to the external surface of the head and thorax, but not in the interior of the body. The remaining pupae did not show any stages of parasites. The five live larvae examined on July 14 were free from the infection. From the above it is seen that three out of thirty-six larvae were heavily infected by the Microsporidian and that the infected larvae died in from two to three days in a rearing jar.

Rearing Experiment No. 2.—Seventy larvae out of 184 apparently normal collected on July 10 and taken at random were kept in a glass jar with clean water and bottom. Due to an unfortunate early departure from the place, the observations could not be extended beyond July 14, on which date all the living larvae were examined. On July 13, four were found dead, one of them was heavily infected. Not one of the living larvae examined on the next day contained any recognizable stage of the parasite. In this case it appears that one out of 70 larvae was infected and that 12 pupae from these larvae were free from the Microsporidian.

Eighty-five pupae collected from the water in the boat on July 10 were kept under observation in a glass jar. On July 11, 13 and 14, five, eight and five pupae were found dead. They were not infected by the Microsporidian nor were the sixty-seven adults that emerged from the pupae. It thus appears that none of the pupae or adults examined were attacked by the parasites.

Thelohania illinoisensis in *Anopheles quadrimaculatus*

Six and forty-eight larvae of *Anopheles quadrimaculatus* were collected from a creek at Spring Valley, New York, on August 18 and 21, 1920, respectively. Two individuals from the first and one from the second collection were found to be infected by a species of Microsporidia apparently identical with *Thelohania illinoisensis*. Two of the infected larvae showed a typical opaque white coloration of the body. One of them was so strikingly opaque in color and inactive

that its diseased condition could be diagnosed as due to some microsporidian parasites while it was still in the water. It died in about thirty minutes after capture. In the second infected individual it was distinctly noticed that the adipose tissue had been infected and that the rupture of the membrane of the infected host cells had scattered various developmental stages of the parasite through the general body cavity. The third infected larva did not show any opacity in the body; it however contained numerous young octosporous sporoblasts in the adipose tissue. Three pupae collected from the same place were free from the infection.

No rearing experiments were conducted due to the small number of the larvae that could be collected, but it seemed probable that the Microsporidian multiplies rapidly and as a result kills the host larvae before they transform into pupae.

Thelohania opacita in *Culex apicalis*

Among fifty-one larvae of *Culex apicalis* collected in a small creek at Spring Valley, New York, and examined on from August 11 to 28, three were found to be infected by a new Miscrosporidian for which the name *Thelohania opacita* is proposed. The opaque appearance of the body of some suggested at the time of collection probable protozoan infection. The larvae were kept in different jars as they were collected and the dead ones were examined microscopically as in other cases. The infected ones died on the second, third and fourth days while normal ones died from the third to the tenth days after capture, some having become pupae during this period. Forty-five adults were collected from the surface of the water and from jars; no stages of the Microsporidian could be found in them. Thus it seems probable that the effect of the infection upon the host is lethal and the infected larvae usually succumb before pupation.

The most conspicuous symptom of the infection is a striking opacity of the body. Two of the infected larvae were opaque white throughout; the third was partially infected as shown here (Fig. A). Decrease in activity was also noticeable as in other cases, although no recognizable difference in size could be detected between healthy and infected larvae.

The octosporous sporoblast is mostly rounded, measuring on an average 11.5 by 8.5μ. Its membrane is very delicate, and can be seen only in places as a faint line in fresh condition. It easily breaks up under a slight pressure. Frequently tetrasporous sporoblasts containing spores much larger than those found in octosporous sporoblasts were seen. The two kinds of spores differ only in dimensions, their structure apparently being the same. It seems probable that these spores correspond to microspores and macrospores of other species

reported by several European authors (Kudo, 1916). I do not find any evidence which might justify the view presented by Vaney and Conte (1901) concerning the significance of these two kinds of spores. On the other hand, the statement I made in a former paper (Kudo, 1916) appears to be supported by the present species. In my opinion the nucleus of the sporoblast ordinarily divides three times producing eight nuclei, while under certain conditions, the nucleus divides only twice producing four nuclei. The former sporoblast gives rise to smaller and ordinary spores, while the latter gives rise to larger and abnormal spores.

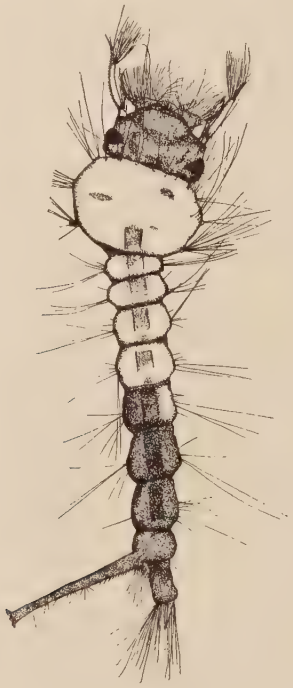

Figure A. — Dorsal view of larval *Culex apicalis* infected by *Thelohania opacita,* drawn soon after being killed with hot sublimate-alcohol-acetic acid mixture. The entire thorax and anterior four segments and a part of the fifth segment of the abdomen, show the characteristic appearance due to the presence of the parasites. × 11.

The spores are broadly ellipsoid and are circular in cross sections. One end is more rounded than the other. At the former extremity a clear space is usually present in mature spores. This space is rounded triangular, oval or circular in shape. The rest of the contents of the spore is uniformly and finely granulated. Dimensions: normal spores, 5.5 to 6μ long by 3.5 to 4μ broad, length of polar filament extruded by

mechanical pressure 110μ; abnormal spores, 8 to 8.5μ long by 4.5 to 5.5μ broad (an exceptional one reaches a size of 10 by 5.7μ); length of polar filament 200μ.

The full morphology and development of the species will be considered in later papers.

SUMMARY

1. Microsporidian infection often is lethal in its effect upon the mosquito larvae.

2. In captivity infected mosquito larvae die in much shorter time than uninfected ones.

3. Pupae and adults so far examined are free from the infection.

4. A new Microsporidian, *Thelohania opacita,* parasitic in *Culex apicalis* is described.

5. The geographical distribution of Microsporidia parasitic in mosquitoes is shown.

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PHASES IN THE LIFE HISTORY OF A HOLOSTOME, *CYATHOCOTYLE ORIENTALIS* NOV. SPEC.,

WITH NOTES ON THE EXCRETORY SYSTEM OF THE LARVA *

ERNEST CARROLL FAUST

Adult holostomes have been known for many years, while the tetracotyliform larvae have been figured in many of the early works on helminths. Since the investigation of Ercolani on *Tetracotyle typica* (1881) the genetic relationship of larva to adult has been understood, but the remainder of the life cycle has been in doubt. Most helminthologists have regarded the life history as monogenetic, although adequate evidence on this question has not been advanced. I have shown in a previous paper (Faust 1918: 15) that in one species, *Tetracotyle flabelliformis,* the larval holostome arises parthenogenetically within a redia, supporting the view of digenetic development. Moreover, the embryo of *Strigea (Holostomum) cornucopiae* figured by von Linstow (1877) and Brandes (1890) is, without doubt, a miracidium, so that the only part of the life cycle lacking experimental proof in one or another holostome species is that bridging the gap between the miracidium and redia.

Furthermore, the homologies of the holostome group have been comprehended by few investigators, mostly because they have not traced fundamental structures from larva to adult. However, Odhner has shown a keen insight into the relationship of the group and to him belongs the distinction of clearing up many doubtful points.

It is hoped that the study I have recently had an opportunity to pursue will serve to make the group of the holostomes better understood along certain lines.

Tetracotyliform larvae were obtained during the spring of 1921 from the testes of the lesser edible snail, *Vivipara lapillorum* Heude, secured from the North Lake of the Imperial City, Peking, and from the Erh Châ Canal outside the southeast corner of the Tartar City. In all nearly seven hundred snails were collected of which 10.3 per cent. harbored these holostome larvae. The same hosts were also infected to a lesser degree with a cystocercous cercaria, *C. pekinensis,* echinostome cercariae, xiphidocercariae, monostome cercariae, a cercarieum species and aspidobothrids. All of the holostomes obtained

* Contribution from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.

were in the encysted condition. In some instances only a few were obtained from each infected host; in other cases several hundred were secured. In one host the liver gland as well as the testis was infected.

Description of *Tetracotyle orientalis* nov. spec.

The encysted tetracotyle measures 0.4 to 0.5 mm. in length by 0.3 to 0.4 mm. in transverse diameter. It is pyriform and is covered with minute spines over the anterior half of the body. I have not been able to dissect away the cyst wall without injuring the larva, but the internal structure has been readily studied through the capsule when the specimens were compressed under a cover glass.

In the uncompressed condition the animal is very active, moving freely within the cyst wall. On the ventral side there are, in addition to the oral sucker, several conspicuous structures typical of the tetracotyle. The oral sphincter measures about 45 μ in diameter. Somewhat posterior to it is a pair of lateral suctorial grooves, which consist only of auricular depressions in the body and lack true sphincter muscles. Slightly posterior to these is a large pouch extending to the subdistal region of the body. In longitudinally contracted specimens the anterior margin of the pouch canopy all but forms a straight line at right angles to the long axis of the worm. At other times its lateral ends almost meet anteriorly, constituting the broken circumference of a circle. Just under this canopy on the floor of the pouch in the midline lies the functional ventral sucker, subequal to the oral sucker. Behind it lies the ventral suctorial apparatus (Fig. 1), consisting of a divided anterior lip and a single posterior lip. I have shown for another species, *Tetracotyle iturbei* (Faust 1919: 71), that the functional ventral sucker is the primitive genital pore, whereas the posterior ventral suctorial apparatus is the homolog of the true ventral sucker of distomes. While the present study reveals no such striking homology, the anterior and posterior ventral suctorial structures may be regarded in the same light.

Internally, the digestive apparatus and the excretory system have been studied in living material while the beginnings of the genital organs have been observed in stained mounts. Within the oral sucker there is a small pharynx, 15μ in transverse diameter. Behind this is a short well-defined esophagus. The ceca are simple and extend backward in a broad bow only as far as the ventral suctorial apparatus.

The genital organs are retarded in their development. In many tetracotyliform larvae from mollusk hosts the organs can be readily differentiated. In this species only the vitellaria have become separated from the germinal cells and have come to occupy a position lateral to the lateral suctorial grooves. The remaining organs still lie in the

genital complex posterior to the ventral suctorial apparatus, with a longitudinal cord of cells extending to the posterior margin of the worm.

The Excretory System in the Larva

The excretory system of the holostome larva consists of two parts, the bladder and the tubule system with the flame-cells at the termination of the capillaries. Odhner (1913: 312) correctly described the former (the reserve bladder) as a close subcutaneous network of relatively wide canals. These are usually recognized by the granules which occupy the canal system and which are pushed back and forth along the canals by the movements of the living worm. Each described species has a fairly unique pattern of such granules, although those of *Tetracotyle flabelliformis* and of the species here described show several points of resemblance (Fig. 4, right half). In many of the described tetracotyles it has been impossible to distinguish between the granular-canal system (the reserve bladder) and the capillary-tubule system which constitutes the primary excretory apparatus. The latter is seemingly very complex, albeit mathematically exact, and is frequently concealed by the superficial network of granules. The few flame-cells which can be seen at times in most forms give no true conception of the real pattern of the system while an attempt to trace them out leads one to see the difficulties in the study.

In the case of *Tetracotyle orientalis,* there was abundant material for study which was constantly available through more than two months of the spring. This enabled me to select only those specimens for study of the flame-cells which contained a minimum amount of granules, but even then the apparent complexity of the flame-cells required many hours of patient work to explain. But when the system was actually unfolded its real simplicity and the precision of the divarications were immediately evident (Fig. 4, left side).

The bladder *(sensu stricto)* consists of a short median portion with a pair of cornua. From the anterior end of each cornu there arises a primary tubule which extends anteriad close to the lateral margin of the worm. Five secondary tubules are given off at graduated distances along the primary tubule. Each of these latter subdivides into equal dorsal and ventral portions. Each portion, too, has a five-fold dichotomy. This provides the dorsal and ventral portions of each side of the body with 160 flame cells, whereas each one of the five main tubules drains 64 flame cells. The entire system, therefore, consists of 640 flame-cells.

Since the system when fully known is so readily reduced to a simple formula, I suggest that it be designated as $\alpha + \beta + \gamma + \delta + \epsilon$, corresponding to the symbols used for the basic excretory apparatus in my previous papers. While further subdivision of the capillaries

and flame cells may occur as the larva grows into the adult, studies on the excretory system of trematodes have now arrived at a point that one may predict with reasonable assurance that, in such instances, the sum will be a multiple of the least common denominator, in this case, $\alpha + \beta + \gamma + \delta + \epsilon$.

Furthermore, it is evident from studies I have made of many living specimens of this and other species that the network of granules is only a reserve system, which is extremely useful in the encysted larva when the capsule prevents ejection of the excretory material, but which is not in use in the free-living post-cystic condition.

Experimental Data on the Life History

During my study of the holstome larva, I attempted feeding experiments on young Chinese domestic ducks with the idea of testing out whether these larvae could continue growth in this type of host.

Altogether, 6 young ducks were obtained. They had never been near any ponds or canals and had been fed only on a mash consisting of cooked millet and cooked cabbage leaves. They had been raised by hand in a mat basket. When about one month old they were brought to the laboratory. They were fed daily on a similar diet of cooked millet and cooked cabbage leaves. For a period of ten days they were observed and their feces examined daily for ova. On April 1, 1921 one of the ducklings, at that time one month old, was infected *per orem* with about 200 encysted *Tetracotyle orientalis* and a few encysted echinostomula. Four of the other ducks were infected with other larval trematodes, while one was kept as a check. The diet of millet and cabbage was continued, and the feces examined daily. On the twelfth day large trematode eggs were recovered from the feces of the duck experimentally fed with holostome larvae, while the other animals remained negative. The positive animal remained in good health until the seventeenth day, when it developed a case of acute diarrhoea and died within a few hours. Examination of the diarrhoeic stool showed several of the same large eggs, while the autopsy of the animal showed several immature echinostomes in the small intestine and seventy-five small holostomes in the ceca. The holostomes were closely applied to the cecal mucosa which was blood streaked, while the lumen was filled with a purulent fluid.

It is my belief that the worms were responsible in part for the death of the host. All the other ducks were negative up to the time of their death which occurred after that of the positive animal and on autopsy showed no infection.

Examination of the holostomes recovered showed that about half of them were mature, with one or two eggs in the uterus, while the others were less advanced. A study of the adult specimens has proved

them to belong to the genus Cyathocotyle Mühling 1896. They cannot be allocated to the described species of this genus, and for them I suggest the name *Cyathocotyle orientalis.*

Description of *Cyathocotyle orientalis* nov. spec.—The mature worm is pyriform and measures from 0.7 to 1.3 mm. in length by 0.5 to 0.9 mm. in width (Fig. 3). The body is covered with small spines in the anterior half. The lateral view has the appearance of a short cucumber, except for the large suctorial cup on the ventral side. The worm is readily recognized as a holostome because the genital ducts open into a posteriorly located genital atrium. *Cyathocotyle orientalis* has an oral sucker which is 110 μ in diameter. It leads directly into a pharynx 45 μ in transsection. From it there proceeds a short but unmistakable esophagus such as Odhner (1913: 312) has shown to obtain for this group. The ceca constitute a graceful bow which extends to the subdistal region of the body. They consist of large epithelial cells with glandular contents.

The most noticeable feature of the mature worm is the genital system, and of this system the two testes are the most conspicuous elements. They consist of a smaller oval body ($350 \times 230\ \mu$) situated anterior and sinistral to the mid region of the worm, and a larger similar body ($480 \times 270\ \mu$) to the right and posterior to the midregion of the worm (Fig. 3, *T*). A pair of short slender vasa efferentia arise from them and proceed to the very short vas deferens which opens immediately into the coiled vesicula seminalis. This merges with the large cirrus sac, which contains a long penial organ. The latter may be protruded as much as 100 μ outside of the genital chamber. The vesicula seminalis is filled with spermatozoa. No prostate glands have been observed.

The ovary is a small ovoid body lying anterior to the right testis. It opens by a short oviduct, which joins Laurer's canal as it proceeds to the ootype. There is a receptaculum seminis which exists as a lateral outpocketing of Laurer's canal for the storage of spermatozoa. In this respect I agree with Mühling (quoted by Odhner, 1913: 309) in finding a seminal receptacle and must, therefore, differ from Odhner himself who regards the contents as similar to the flame-cilia of the fertilization-room of the oviduct. The contents are long, coiled and stain similarly to the contents of the vesicula seminalis. The vitellaria of the adult develop from the two masses of finely divided materials differentiated as vitelline glands in the tetracotyle. In the adult, however, they are aggregated into discrete glands packed with vitelline material. A study of the immature worm (Fig. 2) shows a transition stage in which these vitelline elements have assumed definite lobulations but have not yet become discrete. Fine ducts connect them with the common vitelline duct which leads into the ootype from the anterior aspect.

The ootype is surrounded by an encircling mass of glands. The naked ovum constitutes a very small part of the egg. The greater portion of the latter consists of yolk cells. The uterus is an uncoiled tube leading from the ootype directly to the genital atrium. I have not observed more than two eggs in the uterus while most of my specimens contain only one. The uterine egg measures about $100 \times 65\ \mu$, which measurements differentiate it from the two described species.

DISCUSSION

In an examination of thirty-two domestic ducks in Peking, Changsha, Wuchang and Kuling I have found no members of the genus Cyathocotyle and only one of the genus Strigea. There is still need of evidence, then, to show that the Chinese domestic duck is the natural host of *Cyathocotyle orientalis*. It does seem clear, however, that this duck can harbor the parasite until maturity and that the parasite when lodged in the cecum of the duck acts as a pathogene. While these experimental data are important in showing the relationship of parasite to larval and definitive host and the method by which infection of the definitive host may be accomplished, they are considerably more significant in demonstrating the relationship of the genus Cyathocotyle to the holostome group. Ward (1918: 409) refers to this genus as being "without differentiated regions," a statement which is true in the light of our usual conception of the holostomes, where there is ordinarily found a posterior region conspiculously set off from the anterior portion, the former containing the full complement of reproductive organs.

Odhner (1913) has shown that Cyathocotyle is fundamentally a holostome, although in many ways it partakes of distome characters. A comparison of the tetracotyle larva (Fig. 1), the transitional stage (Fig. 2) and the adult (Fig. 3) of *Cyathocotyle orientalis* indicates how many of the superficial characters may be modified in development from larva to adult. These changes are particularly embodied in the ventral suctorial apparatus. In the tetracotyle there is the normal complement of tetracotyliform suctorial structures. In addition to the oral sucker there are the lateral suctorial grooves, the hood and the functional ventral sucker, back of which is a typical set of paired anterior and single posterior suctorial lips. Careful inspection shows that the lateral suctorial grooves and the hood of the ventral pouch are merely the simplest kind of invaginations of the integument and superficial musculature and are not provided with special sphincter muscles, such as, for example, the lateral suctorial grooves of *Tetracotyle flabelliformis* (Faust 1918). In the transition to the mature worm these lateral depressions entirely disappear, which condition obtains even in the immature form as shown in fig. 2. As for the hood of the

pouch, it, too, becomes less conspicuous, while the posterior suctorial labia become resorbed. These latter changes are accompanied by a considerable enlargement of the functional ventral sucker, which comes to occupy the entire region of the ventral suctorial pouch, taking upon itself the structure and function of a powerful muscular sucking cup. In the immature worm the vestige of the ventral pouch is still apparent, but in the adult (Fig. 3) it has entirely atrophied. A most unique phenomenon has, therefore, resulted, namely, the complete disappearance of the original (posterior) ventral suctorial apparatus and the assumption of this structure and function by the anterior organ which is phylogenetically the genital pore (Faust 1919: 72).

After studying the literature on the described tetracotyliform larvae, I believe the data are too meager and the characters too poorly defined to predict genetic connections between larvae and adults on the basis of described characters. It seems, clear, however, that the larval group designated as "tetracotyles" belongs to several genera, among which are Cyathocotyle, Strigea, and probably Prohemistomum. While the usual method of transfer of the holostome larva is from mollusk directly to definitive host, intercalated secondary larval hosts are known, as for example in the case of *Tetracotyle pipientis,* found encysted in *Rana pipiens* (Faust 1918: 64) and many diplostomula, where the larvae become encysted in the derma and somatic muscles of fish.*

SUMMARY

1. A larval holostome, *Tetracotyle orientalis,* nov. spec. is described from Peking, China.

2. Its excretory system consists of two parts, a network of canals (the reserve bladder) and the system of tubules, capillaries, and flame-cells.

3. The excretory system shows five main tubules on each side of the body, each tubule draining a system of 32 dorsal and 32 ventral capillaries and flame-cells; each of these systems is based on a 5-fold dichotomy.

4. A young duck used for experimental feeding yielded at autopsy 75 holostomes. The controls were all negative.

5. Both immature and adult worms, designated as *Cyathocotyle orientalis* nov. spec., were recovered from the experimental host.

6. Comparison of tetracotyle, immature and adult worm indicates changes involved in the growth of the parasite and relationship of the genus and of the group.

* After this paper was finished an examination of the intestine of a mallard duck, *Anas boschas,* revealed the presence of 268 individuals of *Cyathocotyle orientalis* in the region of the small intestine and cecum. This is particularly significant in view of the fact that the Chinese domestic duck in which the worms were experimentally grown has been bred from the mallard duck.

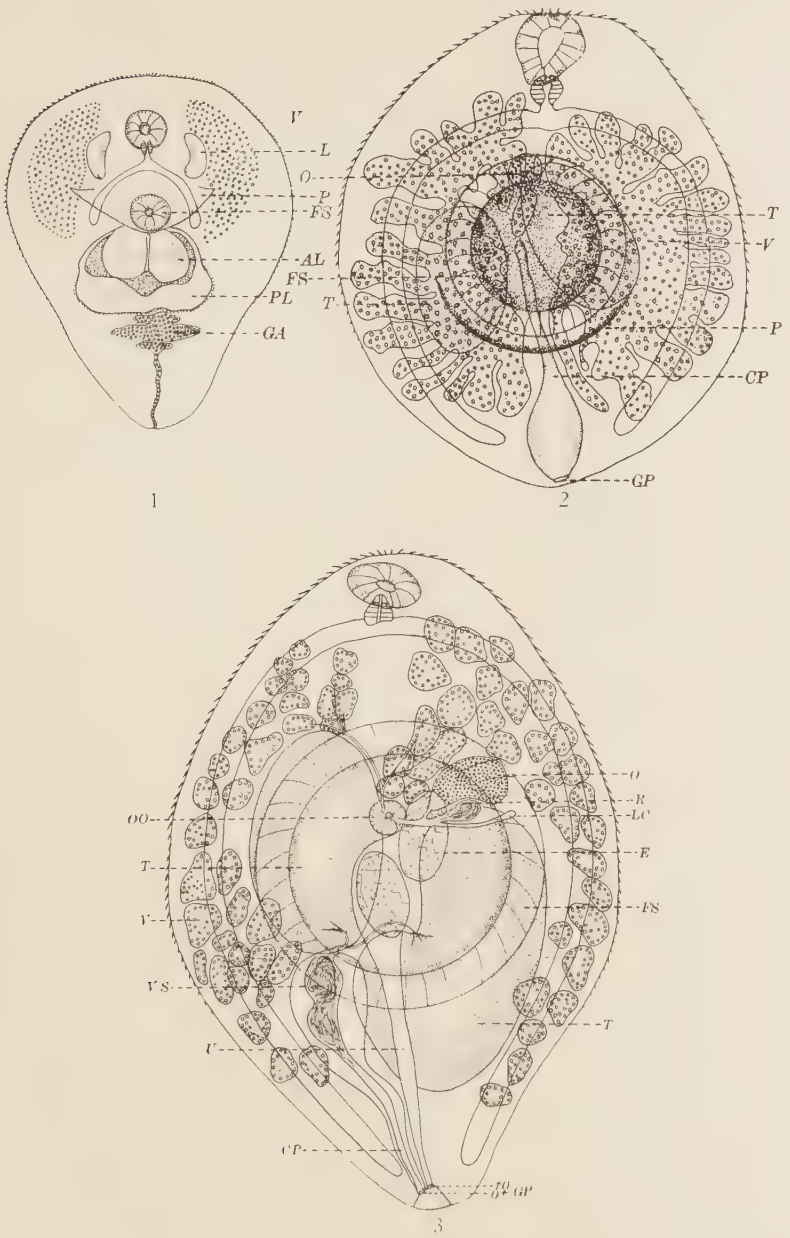

PLATE IX

Fig. 1.—Ventral view of Tetracotyle larva of *Cyathocotyle orientalis*, showing larval organs and beginnings of genital system. × 100.

Fig. 2.—Dorsal view of immature *Cyathocotyle orientalis*, showing vestigial suctorial cup and development of genital organs. × 100.

Fig. 3.—Ventral view of mature *C. orientalis*, showing distribution of adult organs. × 80.

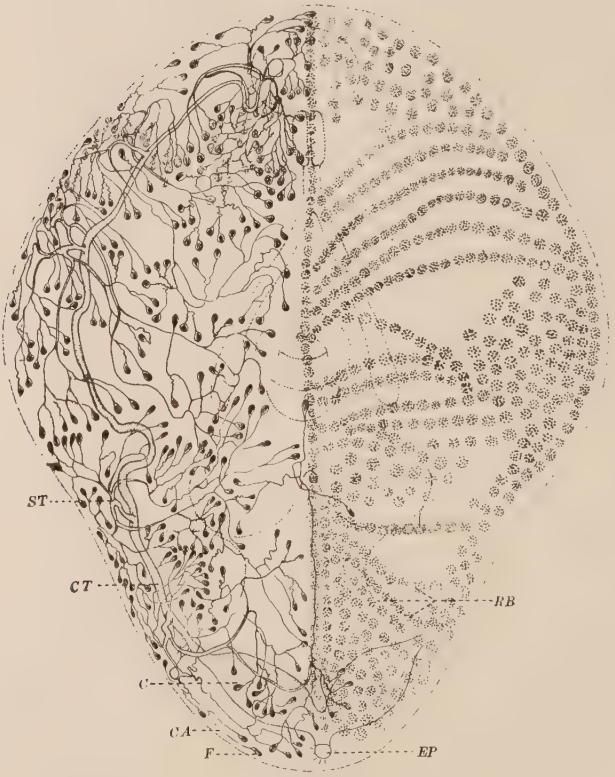

PLATE X

Fig. 4.—Dorsal view of Tetracotyle of *C. orientalis*, showing excretory system: right half, reservoir bladder; left half, tubules, capillaries and flame-cells. ×240.

ABBREVIATIONS

AL—Anterior suctorial lip
C—Cornu of bladder
CA—Capillary
CT—Collecting tubule
E—Uterine egg
EP—Excretory pore
F—Flame cell
FS—Functional ventral sucker
GA—Genital organs
GP—Genital pore
L—Lateral suctorial cup
LC—Laurer's canal
O—Ovary
OO—Ootype
P—Ventral suctorial pocket
PL—Posterior suctorial lip
R—Receptaculum seminis
RB—Reserve excretory bladder
ST—Secondary excretory tubule
T—Testes
U—Uterus
V—Vitelline follicle
VS—Vesicula seminalis

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THE EFFECT OF THE POISON OF TARANTULAS*

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Tarantulas have long been regarded by the general public as decidedly dangerous. In the South, I have found this opinion very firmly established. As an instance of the prevailing opinion, the case may be cited of a man in Texarkana who assured me that his brother was bitten by a tarantula and as a result died on the following day.

These large hairy spiders are fairly common on the stony hillsides near the University campus. Since they can be kept in the laboratory with but little attention, I decided to make some sort of a study of the poison gland and the effect of the venom. In the dissection I used a tarantula that measured 54 millimeters from the upper angle of the chelicerae to the distal end of the abdomen.

The poison gland is located in the basal joint of the chelicera. It is somewhat conical in shape, about 8 mm. long, and measures 2 mm. at its greatest width. The distal end tapers to a point beyond which extends a thin filament about 5 mm. long. This is attached to the very base of the chelicera. The basal portion of the gland is somewhat abruptly constricted to a diameter of less than 1 mm. where it enters the fang. The gland lies in a groove in the musculature of the basal segment of the chelicera and is plainly visible after the dorsal portion of the body wall here has been removed.

The fang is 7 mm. long and about 1.25 mm. at its greatest width. When turned back it fits closely along the inside (mesad) of a row of nine black chitinous teeth. The distal opening of the fang is on the outside curve near the tip.

THE EFFECTS OF THE POISON

The tarantula used for this study was a large female measuring 64 mm. from the upper angle of the basal segment of the chelicerae to the tip end of the abdomen, and 26 mm. across the cephalothorax.

For the first test, a guinea-pig, 7 months old and weighing 635 grams, was used, and the experiment was conducted as follows: The hair on the inside of the right hind leg of the pig was cut off close to the skin. When the leg had been fastened and the pig held firmly, an attempt was made to have the tarantula implant her fangs on the prepared spot.† After several futile attempts on the part of the spider,

**Eurypelma steindachneri* Ausserer, determined by Professor C. R. Crosby, Cornell University.

†As a rule this tarantula will not strike until after considerable agitation. This is contrary to observations made by Petrunkevitch (1911) on *Dugesiella hentzi*.

it became obvious that she could not penetrate the skin. Consequently, the spot having been disinfected with alcohol, a slit one-fourth inch long was made with a small pair of scissors that were also disinfected with alcohol. The slit assumed a reddish color but no blood gathered in it. The tarantula was now placed with both fangs in the incision and by a little agitation was induced to implant them well into the flesh of the pig's leg. A second trial was made and in this, one of the fangs again entered the flesh. The other fang missed the incision and was therefore ineffective. Each time the tarantula struck the guinea-pig squealed, giving evidence of considerable pain.

A number of observations were made on temperature, respiration, etc.; but as all these proved to be obviously of no value they are omitted here. A slight swelling developed in the bitten leg, which probably was due to the incision, but at no time did the pig refuse to use the leg, and in other respects it remained entirely normal.

Another test was made on an albino rat, about 1 month old. The tender skin in this animal proved to be an advantage, for the tarantula had no difficulty in inserting both fangs to their full extent. As in the case of the guinea-pig, the inside of the right hind leg was selected for the test. The tarantula struck twice and the four places where the fangs had penetrated assumed a reddish color, but the blood did not gather in a drop. When the tarantula inserted the fangs the rat squealed and struggled in evidence of more or less pain.

Since the rat showed apparently a definite response to the effects of the poison, the observations are here recorded for August 9:

8:40 a. m.—Tarantula is induced to strike rat twice on inside of right hind leg.
8:53 a. m.—Rat has eyes closed, shows signs of considerable pain, runs about in cage, digs with nose in shavings; leg is held up, seems to be paralyzed.
9 a. m.—Rat has ceased to run, makes sudden jumps as if in response to pain.
9:05 a. m.—Rat almost entirely quiet; now and then a jerky motion of the head.
9:10 a. m.—Rat crosses cage (four feet) in short jumps apparently under grave difficulties.
9:20 a. m.—Rat quiet, seems to be in comatose condition, shows agony when wounded leg is touched.
9:30 a. m.—Rat sleeping, disturbed by sharp pains at intervals.
9:40 a. m.—Rat apparently unconscious, shows no signs of pain.
9:45 a. m.—Rat moving in restless manner.
9:50 a. m.—Rat practically quiet.
10 a. m.—Rat quiet.
10:18 a. m.—Rat moving about constantly, leg held up close to body.
10:40 a. m.—Rat moving very little, sits humped up.
10:53 a. m.—Rat perfectly quiet, lies stretched on abdomen, nose against side of cage.
11:03 a. m.—Rat quiet.
11:25 a. m.—Rat moving about considerably, using the wounded leg in walking.
12:50 p. m.—When lifted rat for the first time opens its eyes wide, seems to be rapidly recovering.
1:45 p. m.—Rat quiet; when picked up acts in a normal manner.
2:45 p. m.—Rat quiet; when aroused appears to be entirely normal.

Later in the day the rat partook of a hearty meal consisting of milk and corn meal.

Acccording to these observations, there is a marked difference between the guinea-pig and the rat in regard to the resistance to the effects of the poison. This is in accord with what Kellogg (1915) cites from the work on the Diadem spider, Epeira diadema, by Hans Sachs.

On the following day, August 10, at 8:55 in the morning, I induced the large tarantula used in the previous tests to strike me twice on the inside of the small finger of the left hand. In the first attempt the fangs barely penetrated the skin. In the second attempt one of the fangs went well under the skin just below the first joint.

The blood gathered enough so that the punctures assumed a reddish color, but did not collect in drops. A small amount of the poison, a clear, colorless, and tasteless liquid was present in all four of the punctures.

The sensation produced by the strike was like that following the stab of a pin. This pain, if it may be called that, decreased gradually till at 9:40 no trace remained. At no time was the finger at all stiff.

On the next day at 9:40 a. m., the experiment was repeated. The large female was again used and with but little agitation was induced to strike twice in about the same place selected on the day before. This time there was a more generous supply of poison than on the day before, with the result that two large drops collected and ran down on the sides of the finger. In one of the punctures a small drop of blood gathered; the others merely assumed a reddish color.

The pain was very much as was described in the previous test; at first fairly sharp, then gradually becoming dull till at 11:40 all traces of it had disappeared.

From the description of the tests on myself as given above it is easily seen that I did not get what would be considered a full dose of the poison. The experiment therefore does not show what would happen in a case where a person was bitten so that the full dose of the poison entered the blood stream. Nevertheless, I believe that normally the bite of a tarantula is not dangerous to man, and that even a full dose of the poison would probably not produce any very serious results is indicated by the observations on the rat. There is no doubt in my mind that the rat got all the poison that the tarantula had available at the time.

It should be added that in the tests on myself I did not resort to the use of any disinfectants; in fact the punctures were not tampered with in any way.

With regard to relative susceptibility to insect poison, I would probably be considered an average individual. The sting of a bee causes moderate swelling and a sharp pain that lasts for ten or fifteen minutes.

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AN UNUSUAL FORM OF SCABIES IN FOWLS*

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In the course of investigations dealing with the parasitic infestations of poultry during the summer of 1919, a condition of rare occurrence was observed in two cockerels forwarded to the Biological Laboratory for examination, and which, because of its rarity, is probably worth recording.

The fowls in question were forwarded to us from Oka, La Trappe, Province of Quebec, and upon arrival presented the following symptoms: Inability to eat owing to the eyelids being completely glued together; heads held down in a rather pendulous state, the comb, wattles, eyelids and face being covered with dried exudate and epidermal crusts somewhat similar to those seen in newly recovered cases of chickenpox or a mild form of infra-orbital roup.

These fowls were placed in experimental coops pending the receipt of information relative to the suspected condition, and upon examination two days later were noticed to be much improved, the eyes being open and the birds eating ravenously.

As no detailed information had been received, these cockerels were killed on Oct. 30, 1919, for internal examination, as it was thought probable that tapeworms were present in the intestines, and it was then noticed that the legs were partially devoid of scales. The scales had exfoliated, leaving the shafts of the legs rather whitish in color, the denuded areas being in sharp contrast to the normal yellowish scales still present. One cockerel showed a larger area of desquamation than the other bird.

The condition did not in any way resemble the ordinary form of Scaly-leg due to the mite *Cnemidocoptes mutans,* no large calcareous crusts being present, nor deep cracks or erosions. Furthermore, this parasite could not be found although an extended search was made at the time.

In scrapings from the heads and legs of these cockerels a parasite, new to us, was discovered to be present in large numbers. This parasite, through the kindness of Dr. F. C. Bishop, Bureau of Entomology, Dallas, Texas, and Dr. Ewing, Washington, D. C., was identified as *Megninia gallinulae* Buchh.

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* Published with the approval of Dr. F. Torrance, Veterinary Director General.

Outline drawings of male and female parasites are herewith reproduced, but satisfactory drawings of larvae could not be made owing to excessive shrinkage after mounting, although many were present in the scrapings.

Megninia gallinulae—male.

Megninia gallinulae—female

INFECTION EXPERIMENT

On Oct. 31, 1919, three yearling Barred Rock hens were procured for experimental purposes. These hens were in the period of moult, but otherwise were quite healthy. The heads were rubbed freely with a small brush until the serum commenced to exude slightly and scrapings containing parasites were then rubbed into the scarifications. A careful daily observation was made of these fowls and no untoward effects were noted, the hens continuing to moult normally and recovering their new feathers in the course of a month's time.

On Jan. 30, 1920, these hens were removed from the experimental coops and scrapings from the heads and legs failed to show evidence of any parasites.

As previously stated, the only information available as to the point of origin was the express label bearing the inscription, Oka, La Trappe, and although several attempts were made to ascertain the name of the consignor, and the extent of the infection, all our inquiries remained unanswered.

It is unfortunate that our suspicions as to the exact nature of the disorder were not earlier aroused, as the mating of these cockerels with young pullets might have resulted in a natural infection.

SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The fifty-first meeting of the society was held on March 17, 1921. Dr. R. T. Leiper of the London School of Tropical Medicine gave an informal talk, noting especially that he had reexamined Cobbold's type specimens of *Distomum coronarium* and found this fluke to have a structure which, so far as known at present, is unique among trematodes. This structure consists in intestinal ceca opening to the exterior of the body. In all other known flukes the ceca end blindly or open into the excretory vesicle. An examination of 12 specimens of this species, collected at the London Zoological Gardens, confirms the observation made on Cobbold's material.

In a discussion of tropisms, Dr. Leiper noted that the egg-laying habit of the Guinea worm was not in the nature of a hydrotropic response. In Loeb's work thermotropism was found to be unimportant. However, Leiper finds thermotropism apparently involved in the invasion of the skin by hookworm larvae. In tests of thigmotropism, it was found that hookworm larvae came through filter paper only in the last few drops of water. When the larvae were under a cover glass at the bottom of a preparation and the cover glass was heated, the larvae came up to the warm areas. Similarly, when a heated coin was put on one end of a slide on which there were larvae in a drop of water, the larvae quickly went toward the coin, even leaving the fluid and becoming stranded on the dry slide. Free-living nematode larvae do not react in this manner.

In connection with control measures for schistosomiasis, Dr. Leiper noted the importance of measures against snails. Mollusc eggs are quickly killed by drying. Chandler has recommended the use of copper sulphate solution against snails, a 1:1,000,000 solution killing them in 24 hours. This measure is applicable in controlling intermediate hosts of *Schistosoma*. The passage of an electric current between two pieces of copper foil in water will produce poison enough to kill snails. *Planorbis boissyi* and *Bullinus contortus* can be killed by 1:5,000,000 solutions of copper sulphate, but *Limnaea truncatula* requires a strength of 1:2,000.000. Tartar emetic will also kill snails, as Christopherson has shown. Leiper, however, is unable to confirm the statement that tartar emetic will kill the miracidia of blood-flukes, even when used in saturated solution. Apparently its action in therapeutic use is indirect, not direct.

Dr. Leiper also discussed the passage of schistosome eggs through tissues. It appears that there is an actual migration, rather than the mere passage of inert foreign bodies. After treatment with tartar emetic, these eggs come away very slowly, the contained embryos are dead, and the eggs do not cause symptoms, as live eggs do. The live eggs are not impacted in the tissues, they cause ragged edges along their paths, they pass blunt end first and the miracidia may be pointed back along their paths. It appears probable that their passage is due to a secretion. The miracidium is provided with two large glands in the anterior end of the body, and it appears that these may secrete a substance which erodes tissue as that of the glands in the cercaria does when the cercaria enters its host. It is believed that these glands function in the penetration of the tissues of the secondary host by the miracidium; it seems probable that they function in penetrating the tissues of the primary host as the egg escapes. However, in view of what is known in regard to some other flukes, Leiper doubts whether the glands function in the entrance into the body of the secondary host.

In comment on Dr. Leiper's remarks, Dr. Cort noted that he had submitted some slides showing the passage of schistosome eggs through tissue to Dr. Simon, who reported that the effect produced by the eggs could not be attributed to

the mere action of a foreign body, producing, as they do, severe reactions and eosinophilia. The movements of the eggs did not accord with those of inert foreign bodies and he was confident there was a toxin present. In answer to a question, Dr. Leiper stated that the eggs did not take a direct path toward the lumen of the involved organ, and that apparently the spine, which points backward along the path, serves to prevent the eggs going back and not to force or cut a passage.

Dr. Cort noted that when a concentrated light was turned on a culture of hookworm larvae in a Syracuse watchglass, the larvae quickly went toward the illuminated area. This had been regarded as a phototropism, but apparently, in the light of Dr. Leiper's remarks, it should be interpreted as a thermotropism. In comment, Dr. Leiper stated that the larvae never rise to the surface of a fluid under the influence of light alone.

Dr. Ransom noted that the larvae of *Haemonchus contortus* ascend the sides of a glass container and descend from time to time as conditions change; Veglia has thought that this is a heliotropism, but it may be a thermotropism. Dr. Cobb called attention to the fact that free-living nematode larvae commonly float on water and that one form parasitic in insects also floats. Some of these larvae behave as an oiled needle would and are very difficult to sink.

Dr. Bartsch stated that collectors of molluscs always carry copper sulphate, and that he has personally used it in many places in collecting. If a pint of a saturated copper sulphate solution is poured in a tide pool the size of an ordinary room, everything that can leave does so, including all sorts of worms, octopi, etc. Fish come to the surface. In fresh water, the solution does not act as effectively. Incidentally, he noted that an excellent method for collecting snails was to rub some suet on the under surface of a plank and leave it for a day or so, at which time it will be found to have attracted large numbers of snails. The suet may also be dragged over freshwater algae and water plants and in a day or so numerous snails will collect along its path.

In reply to a question, Dr. Leiper stated that a 1:1,000,000 solution of copper sulphate will kill algae and that a 1:1,000,000 solution will kill some small fish, such as "millions," the mosquito-control fish. Kobayashi says the solution interferes with rice culture, but Dr. Leiper stated that Kobayashi used too strong a solution. He stated that the solution was believed to interfere with the action of ferments and was a heart depressant. In this connection, Dr. Bartsch noted that from his observations on copper sulphate solution action on fish it would appear that the chemical interfered with respiration.

Dr. Bartsch reported that a case had come to his attention of an infestation with *Taenia saginata* in a 6-months-old child that had been fed on milk alone. In comment, Dr. Ransom suggested that there were several ways in which this might be accounted for, such as the use of a knife with which infested meat was cut, whereby a cyst might in some way be transferred directly or indirectly to the milk.

Dr. Ransom reported a *Syngamus* from the respiratory tract of a calf in Porto Rico resembling, if not identical with, *Syngamus laryngeus* heretofore known only in the Orient. Dr. Bagué, who sent in the specimen, reported that the calf had a verminous bronchitis.

Dr. Cort presented an abstract of a paper by Yokogawa on the life history of *Heligmosomum muris*. A bagging of the cuticle over the posterior end in the adult female represents the unshed cuticula of the previous molt. The free-living larva molts only once and is then infective. The larva penetrates the skin, goes to the lungs inside of 24 hours, molts in the lungs, and then goes to the digestive tract. After a third molt in the digestive tract, the worm has practically attained maturity. No fourth molt occurs, the worm retaining the last cuticula, which is attached anteriorly but loose posteriorly, especially in the female.

Dr. Cort also presented a note on the development of apparatus for recovering hookworm larvae from soil on a large scale. An apparatus used by a Dutch investigator in work in Batavia, Java, has been modified for the purposes of the expedition to Trinidad. A large brass funnel, 7 inches in diameter,

has a heavy rubber tube attached to the narrow end and a pinch cock applied to this tube. After the funnel is filled with water, a sieve is fitted in at the surface of the water. A cheesecloth layer is inserted to prevent the passage of dirt, and the dirt placed in the sieve. An hour later the pinch cock is opened and the larvae drawn off. The Dutch worker in Java says he could recover 1 of 2 larvae placed in his apparatus. With the modified apparatus, Mr. Ackert has recovered over 400 larvae of 620 placed in a pint of dirt. In the work in Trinidad, 18 funnels capable of handling 1 quart of dirt each will be used. Mr. Ackert noted that of 6 larvae placed in soil, 2 were recovered from 5 cc of fluid drawn off in 16 minutes.

In comment, Dr. Bartsch stated that he believed monel metal would be better than brass for funnels of this sort, and Dr. Cobb also advocated avoiding brass. Dr. Cobb also noted that the jig table appliances used in washing ore might be used to advantage in handling large amounts of soil to be examined for nematodes.

Dr. Ransom stated that some nematodes may have more than the classical 4 molts. In *Strongyloides ovocinctus,* a cuticula surrounding a string of eggs may be shed. Habronema may have more than 4 molts, though this matter needs further study. Some nematodes might have less than 4 molts.

Mr. Ackert presented a note on nematodes in a cricket.

Dr. Barlow exhibited a specimen of a female *Thelazia callipaeda,* a parasite of the eye, which had been collected alive from the feces of a Chinaman after administration of an anthelmintic.

Dr. Cobb exhibited a piece of apparatus for use as a turntable. This consisted of 2 tin pie-plates separated by 3 steel balls on which the upper plate rolled over the lower.

Dr. Cobb also presented a note on the importance of nemas. He noted that *Heterodera radicicola* is unequaled as an agricultural pest. As study material he especially recommended a new species of *Rhabditis* which is easily reared on dead flies. This nema breeds rapidly and Dr. Cobb exhibited over a thousand which had developed from an original pair in the course of 10 days. The worms can be allowed to dry and will rapidly recover activity on being wet again.

Dr. Cobb also demonstrated an apparatus for exhibiting small specimens, the apparatus consisting of a tin container with a Syracuse watchglass containing the specimens at the bottom of the container, a lens attached for viewing the specimens and an ordinary electric flash light set into the top of the container for illuminating the objects to be observed through the lens. Dr. Cobb also noted that when live nemas were put in methyl violet and neutral red, the esophagus stained violet and the intestines red.

The fifty-second meeting was in the form of a dinner on April 9, 1921. The guest of honor was Dr. L. O. Howard.

The fifty-third meeting of the society was held on May 14, 1921.

Dr. Cooper Curtice was elected president, Dr. Hall recording secretary and Miss Cram corresponding secretary-treasurer.

A rising vote of congratulation was extended to Dr. Stiles for the recognition of his work in medical zoology by the recent presentation of the public welfare medal by the National Academy of Sciences.

The death of Dr. James Law, one of the early workers in parasitology in this country, was reported.

Dr. Ransom presented a paper soon to be published (Amer. J. Trop. Med.), by Ransom and Cram, on the course of migration of Ascaris larvae.

In comment, Dr. Stiles noted that medical texts commonly refer to ascariasis as a matter usually of little importance. Such work as that reported by Dr. Ransom shows that this disease is very important. As a rule ascarids in man are less frequent and less important in the city than in the country. In rural districts they may give rise to severe cases resembling hookworm disease, and it may be necessary to differentiate them by resort to microscopic examination of the feces. Both conditions call for the same attention to the proper disposal of excreta. The pathogenic rôle of *Gongylonema neoplasticum* in the production of cancer raises the question as to the possible etiological rôle of

tissue-invading worms in man in the production of pathological conditions that may develop slowly and come to light at a later period when the connection between the cause and effect is not evident.

Dr. Ransom noted that the findings indicate the danger to which children may be exposed in playing about hog lots; even though the identity of the swine and human ascarid has not yet been experimentally demonstrated and though it be assumed as possible that the adult swine ascarids would not develop in children, it is, on the other hand, quite certain that the larval stages would develop in the lungs and other organs. Dr. Bartsch suggested the possibility of using intravitam staining on larvae which were injected into animals for experimental study.

Dr. Cobb presented a note in regard to the federal activities in preventing the introduction into this country of plant pests. In general, horticulturists acquiesce in the federal regulations. The dangers are so numerous that probably the best way to import plants is to import the seeds, which can be sterilized. From material about the roots of *Kentia,* an ornamental plant from New South Wales, 26 species of nematodes were collected, of which 4 species of *Aphelenchus* are new. *Tylenchus penetrans,* previously reported from several plant hosts, was reported from clover and alfalfa in Utah. A specimen of cucumber beetle from Ohio was exhibited, to show infestation with a nema, probably a species of *Aphelenchus.* Of the beetles examined, 30 per cent. were infested. These and similar parasites may have some relation to the decrease of insects following months or years in which they are extremely abundant. Dr. Cobb also exhibited a key to the species of *Aphelenchus* and one to the species of *Tylenchus,* many of the species involved being new.

In comment on the federal control work, Dr. Stiles called attention to the fact that commerce is largely responsible for the spread of pests, parasites and disease, as well as a primary cause of war. Dr. Cobb noted the importance of railroad trains as spreaders of flies, and Dr. Ransom reported some observations in Chicago in the spring of the year when he had found flies present in the railroad trains from the South and apparently not generally present elsewhere in the city. Dr. Stiles called attention to the importance of trains as disseminators of lice.

Mr. Chapin reported the collection from horse manure, in a case where the manure was being examined daily for parasites after the administration of an anthelmintic, of a bot, *Gastrophilus intestinalis,* in the pupal stage. The adult fly, a female, emerged from the pupa in 5 days, a time much shorter than the usual period of 3 to 5 weeks reported for the pupation period of this insect. As this horse was in a stall with a concrete floor from which all manure was carefully collected daily, the possibility that this bot had been passed at a much earlier date in the usual larval stage and had pupated on the concrete floor unobserved seems less likely than that the bot had remained for a long period in the cecum and had pupated there. It has been reported a number of times that bots removed from the stomach by the use of carbon bisulphide, have been found dead after a considerable time (up to 17 days) in the cecum and colon instead of being promptly expelled in the feces.

Dr. Hall reported a second case of the swine kidney worm, *Stephanurus dentatus,* from the liver of cattle. The specimen, a well developed female, was collected by Dr. J. S. Jenison at the National Stock Yards, Illinois, from a 6-year-old cow. This animal was in good condition and the kidneys were normal. The previous case from cattle was reported by Hall at the forty-ninth meeting of the society, the specimens in this case being 2 males collected by Dr. Pote at St. Louis, Missouri.

Dr. Stiles reported the receipt of a collection of hookworms from the dog in the Southern United States in which part of the specimens are *Ancylostoma caninum* and others apparently *Necator americanus.* In reply to a letter of inquiry by Dr. Stiles, the physician who collected the worms writes that there is no possibility of confusion or error in regard to the specimens or the host. Dr. Stiles reported that on a former occasion he had found 1 female hookworm that might be *Necator* in the dog.

Dr. Stiles reported that he had been compiling the literature on chemical means for the destruction of nematode eggs in feces, and now had an extensive bibliography on this subject. Oftentimes it is difficult to determine from the name of the chemical used just what substance is meant. Much of the work is of only academic interest, since the chemical tested is too expensive for general use. It appears that in some soils and some mines the destruction of eggs in feces is accomplished by the natural chemical content.

Dr. Stiles reported that the subject of ground water pollution is to be again investigated experimentally in a selected area by the use of typhoid bacilli, as the previous findings with *Bacillus coli* were distinctly in conflict with generally accepted views and might be objected to on the grounds that *B. coli* is a widely distributed form that occurs rather generally and might be found in tests where it was present from sources other than the material used in testing.

Dr. Stiles also reported tests of gas house tar on the city dump at Mobile, Alabama, for the control of flies. The tar destroyed many flies and drove the others to nearby houses, but its action was transient and after a short time it dried and the flies returned. It would require large quantities to be of service under these conditions and apparently the general use of the tar will only be of service in the case of privies and on similar small areas and quantities to be treated, and not for use on large areas or large quantities. Samples of tar vary widely in composition. The tar is distinctly less effective against flies of the genus *Eristalis* than against those of the genus *Musca*.

In comment on Dr. Stiles' notes, Dr. Ransom noted that *Ancylostoma duodenale* or a morphologically similar form had been reported from swine in the Orient by O'Connor and that *A. braziliense* and *A. ceylanicum*, which species Leiper regards as identical, are reported from the dog and *A. ceylanicum* from man also. He also reported the occurrence of *Uncinaria stenocephala* from the pig in Canada, noting that the common hookworms of dogs in this country is *A. caninum*, but that the hookworm of foxes is *U. stenocephala*. He further reported that he finds *U. polaris* Looss to be a synonym of *U. stenocephala* on the basis of an examination of material of both species. The difference in distribution of *A. caninum* and *U. stenocephala* may be correlated with latitude, *U. stenocephala* apparently ranging north of *A. caninum*.

Dr. Bartsch exhibited a specimen of monel metal in connection with the question of screens and other apparatus for helminthological work and screening against flies. This material, a natural alloy, costs about as much as brass and lasts much longer.

MAURICE C. HALL, *Recording Secretary.*

NEW HUMAN PARASITES

Schistoma bovis (Sonsino, 1876).—In June, 1921, I had the opportunity of watching the effect of a series of intramuscular injections of emetine on the ova of *Schistoma bovis* in a native (South African) boy of 8, who had contracted this infection along with *Schistoma haematobium*. The spindle-shaped eggs were uniformly longer and narrower than the human parasite, and measured from 0.20 to 0.236 mm. in length by 0.06 to 0.07 mm. in breadth. Those of *S. haematobium* were about 0.15 by 0.6 mm. (F. G. Cawston) (Vet. Rec., n. s. 1:817; Oct. 8, 1921). In another paper Cawston (Jour. Trop. Med., 24:242; Sept. 15, 1921) says these ova "indicate infestation either with *S. bovis* or with a larger variety of *S. haematobium* than that which commonly attacks the native and European population of Natal." In 1905, Christophers & Stephens found in the urine of a Madras native ova 205 by 53 μ (Jour. Trop. Med., 8:259) which might have been from *S. bovis*. Cawston's ova were also taken from the urine, whereas the ova of *S. bovis* are discharged only from the intestine so far as is known from previous observations. In the light of this evidence *S. bovis* cannot be listed unquestionably as a human parasite.

BOOK REVIEWS

Le Parasitisme et la Symbiose. By M. Caullery. Encyclopedie Scientifique; Librairie Octave Doin, Paris. 400 pp., 55 text figs.

This interesting volume represents a course of lectures delivered at the Sorbonne in 1919. The topic of parasitism is attacked from the point of view of general biology and as a problem concerning the relations of organisms to each other. The author emphasizes in striking and effective fashion that parasitism, commensalism and symbiosis are not separate categories but a series which in nature is unbroken by any discontinuity. As such a process parasitism becomes the most striking illustration of evolution. The volume is in a way a successor to Van Benden's famous treatise on Commensalism and Parasitism, but the classical illustrations which are presented in detail in that work and have been so widely copied, are here passed over lightly and preference given to later researches and the detailed results that have come from the study of more recent years. The full biography that accompanies the work adds very greatly to its value for advanced students, and the brilliancy with which the topic is presented is characteristically French and sure to attract the general reader who is seeking information in this field.

The Japan Medical World, a monthly which was started in Tokyo last May, has printed several original articles of importance in parasitology. It also abstracts current Japanese medical literature and thus makes available in English the results of investigations in Japan that have previously been almost inaccessible. In view of the extensive and important work on parasites and parasitic diseases done in Japan these reviews, which thus far are exceedingly well written, will be of great value to scientific workers in other countries.

The Journal of the Philippine Islands Medical Association is a new periodical published bimonthly under the direction of the Philippine Islands Medical Association. The numbers thus far issued devote considerable attention to medical zoology. Among the important original articles are papers on parasitology by Haughwout.

NOTES

Jack rabbits, lice, and certain horseflies (*Crysops discalis*) are instrumental in the transmission to man of the infectious disease of rodents now christened tularemia, characterized by *Bacterium tularense* in the blood. Francis reported in 1920 that the disease exists among jack rabbits, to which it seems fatal and from which it is transmitted to man by a blood-sucking horsefly. Later Francis and Lake discovered that the jack rabbits were infested with lice, which spread the disease among the jack rabbits. This explains how the infection is kept alive from year to year in Utah. Tularemia is seldom fatal to man, only one death due to it being known. It is, however, a disabling septic fever, occurring in Utah, which lasts from three to six weeks, and from which convalescence is slow. Its economic consequences may be serious.

The interest of the late Doctor Sir William Osler in Parasitology was evidenced not only by the fact that the subject was given such careful and special treatment in his Modern Medicine when it was briefly and imperfectly treated as a rule in other publications of that date, but also in the number of his own special contributions which bear upon that subject.

In 1874 he described blood platelets often erroneously interpreted before and since as haematozoa. In 1876 he wrote on trichina in an important lecture on Animal Parasites and Their Relation to Public Health; in 1877 he discovered and described the parasite of verminous bronchitis in dogs (*Filaria osleri*); in 1880 it was a hydatid cyst in the liver; in 1882 echinococcus disease in the liver and parasites of the frog's blood; in 1883 cestode tuberculosis and parasites in the pork supply; in 1887 haematozoa of malaria; in 1890 malarial parasites, amebic dysentery and microfilariae; in 1891 multiple cysticerci; in 1895 Laveran's discoveries; in 1899 sporadic trichinosis and blood parasites of frogs; in 1902 amebic abscesses in the liver. These and many other similar items listed among his writings show his constant attention to and interest in the growing field of Parasitology and the significance of these discussions for clinical medicine.

In a recent report the use of carvacrol as a substitute for thymol in hookworm disease is considered by A. E. Livingston of the U. S. Hygienic Laboratory. Thymol is costly and the supply is uncertain. By a recently devised method the supply of carvacrol is assured as a byproduct in the manufacture of wood pulp and at a low cost. Experiments were made on rabbits, earthworms and paramecia; these show that thymol and carvacrol have practically the same toxicity on the animals used. It is recommended that a careful clinical trial be made in hookworm cases where conditions can be fully controlled.